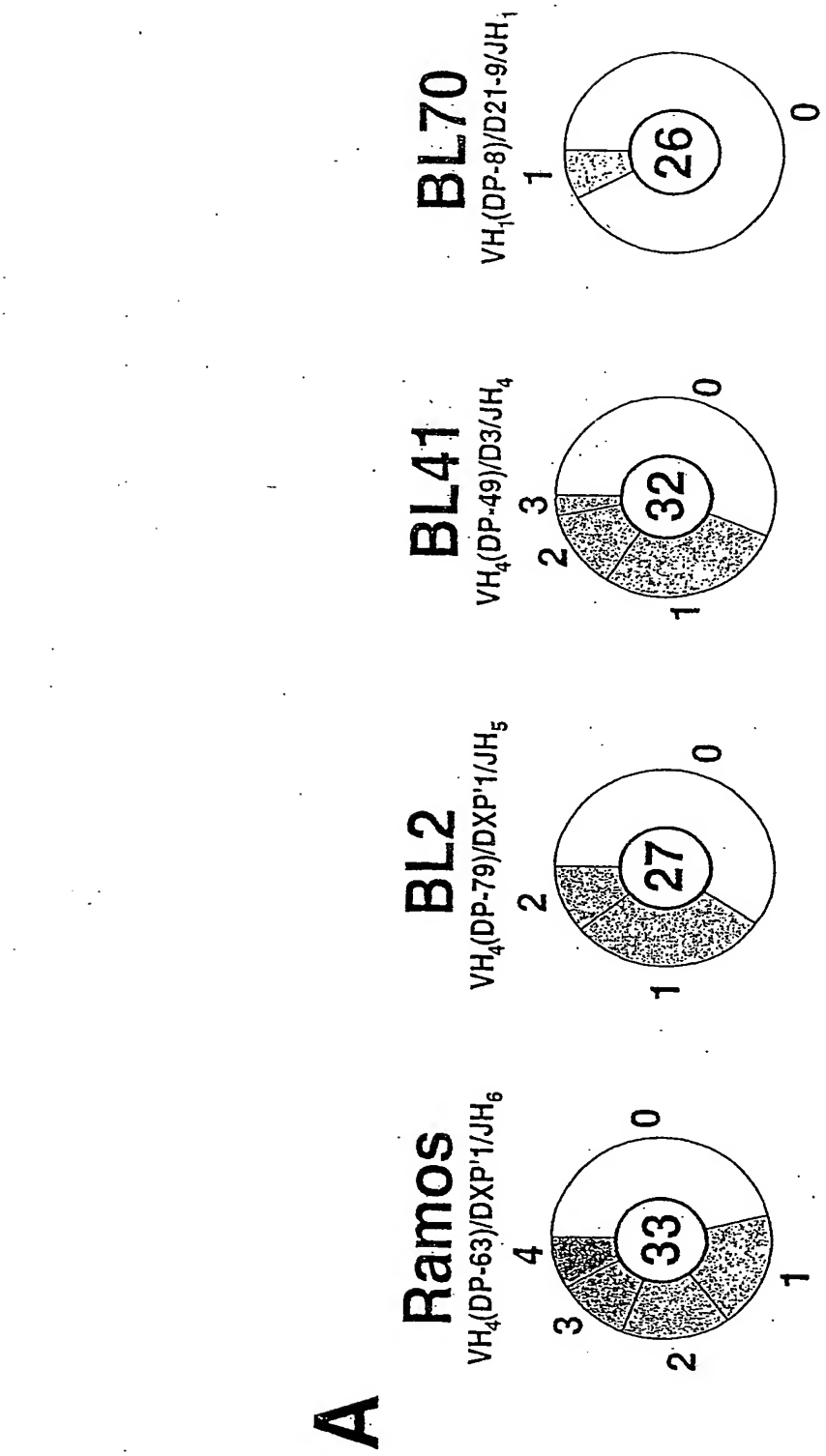

A
Ramos
VH4(DP-63)/DXP'1/JH6
33
0
1
2
3
4
BL2
VH4(DP-79)/DXP'1/JH5
27
0
1
2
BL41
VH4(DP-49)/D3/JH4
32
0
1
2
3
BL70
VH1(DP-8)/D21-9/JH1
26
0
1

FIG. 1

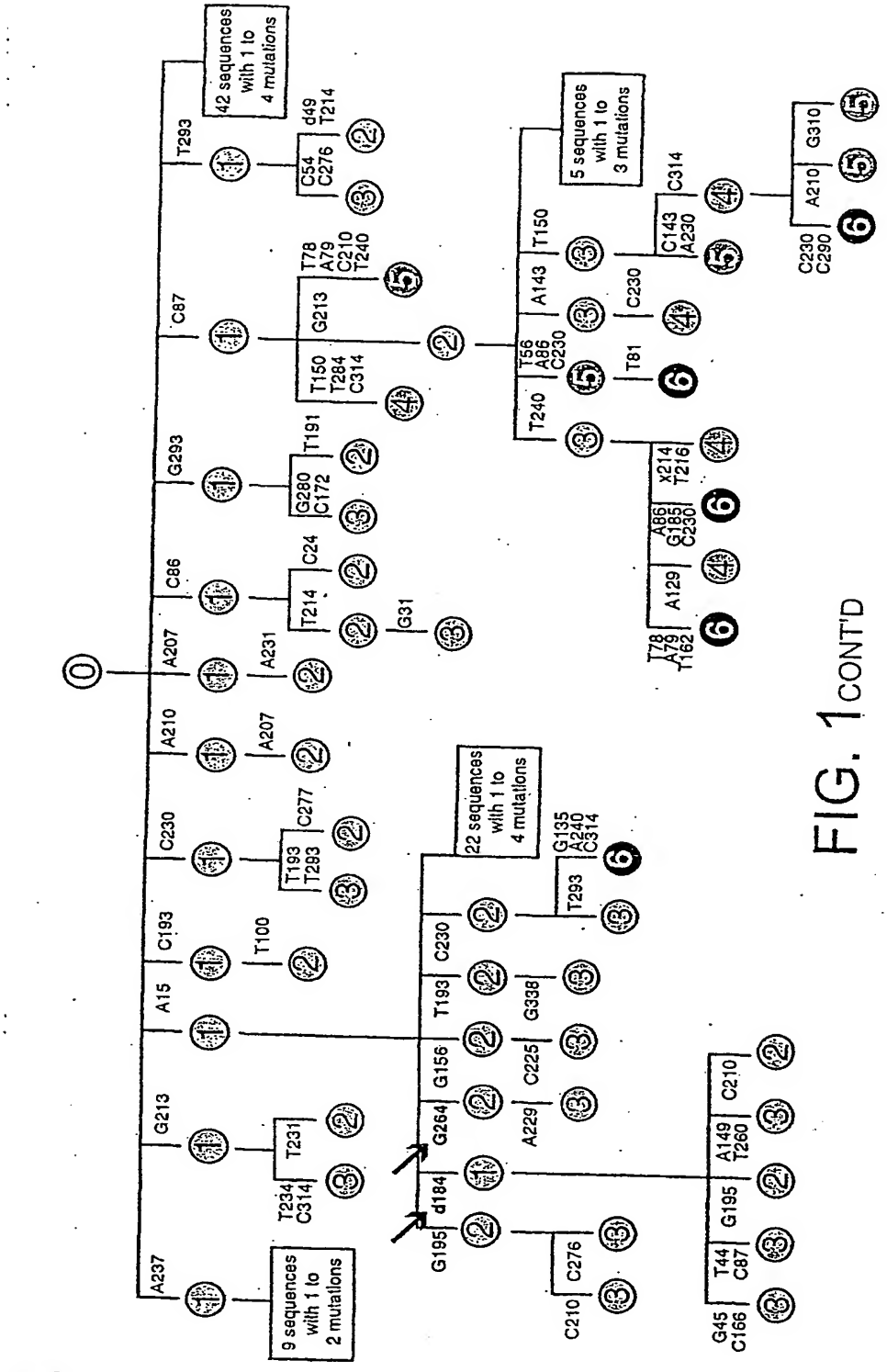
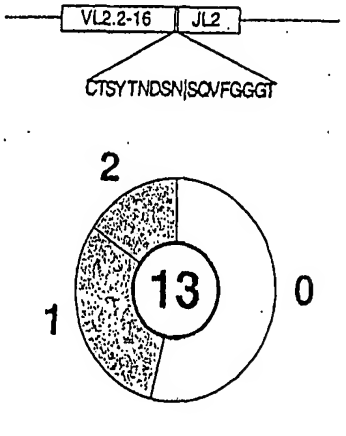

B
0
A237
9 sequences with 1 to 2 mutations
G213
T234 C314
T231
A15
C193
T100
C230
T193 T283
C277
A210
A207
A207
C86
T214
C24
G31
A231
G293
G280 C172
T191
C87
T293
42 sequences with 1 to 4 mutations
C54 C276
d49 T214
G213
T150 T284 C314
T78 A79 C210 T240
T240
T56 A86 C230
A143
T150
C230
T81
C143 A230
C314
5 sequences with 1 to 3 mutations
C230 C290
A210
G310
T78 A79 T162
A129
A86 G185 C230
X214 T216
G195
d184
G264
G156
T193
C230
22 sequences with 1 to 4 mutations
T293
G135 A240 C314
A229
C225
G338
C210
C276
G45 C166
T44 C87
G195
A149 T260
C210

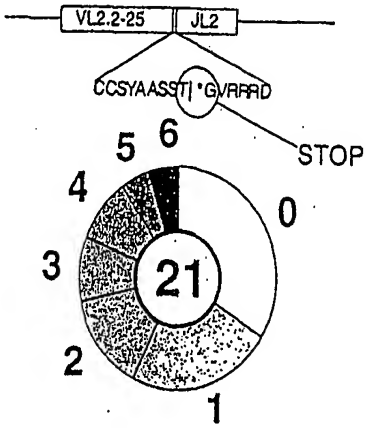
FIG. 1CONT'D

C

In Frame Vλ



Out of Frame Vλ



D

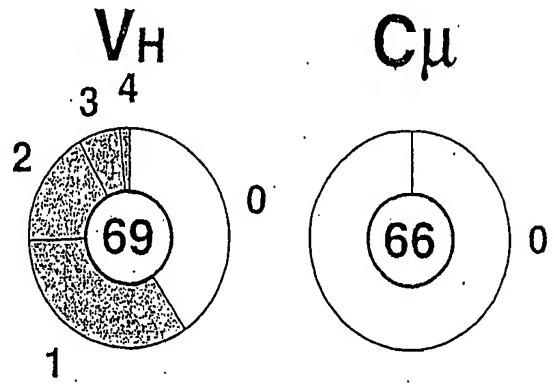
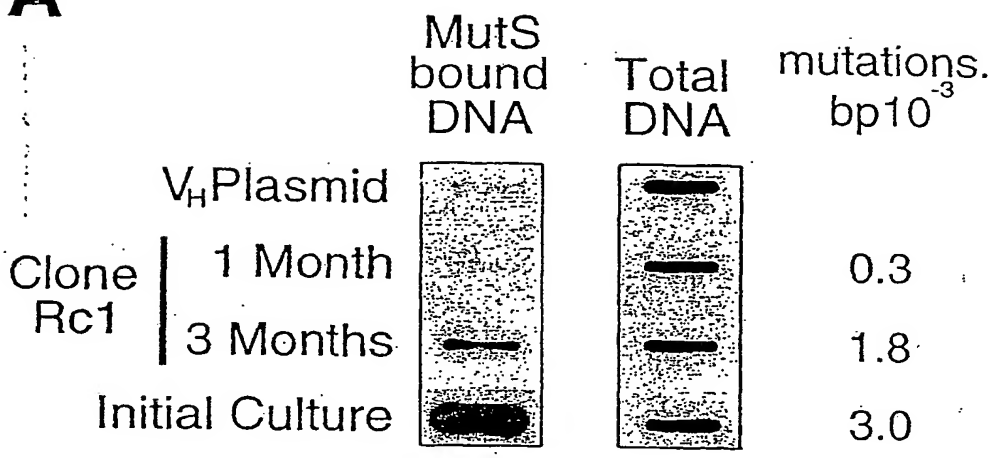
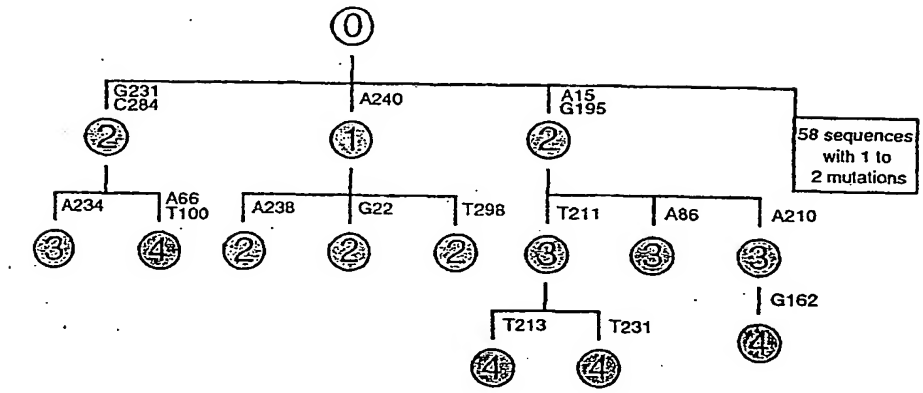


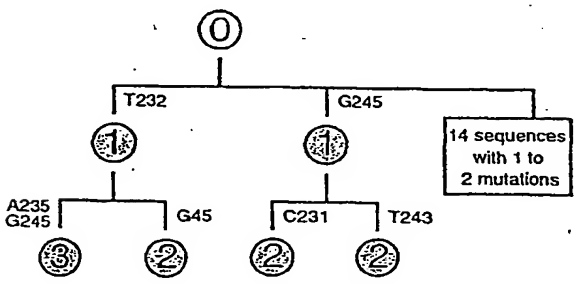
FIG. 1CONT'D

A


MutS bound DNA
Total DNA
mutations. bp10-3
VHPlasmid
Clone Rc1
1 Month
3 Months
Initial Culture
0.3
1.8
3.0

B


Clone Rc13
0.24x10-4 mutn.bp-1.div-1
0
G231 C284
A240
A15 G195
58 sequences with 1 to 2 mutations
A234
A66 T100
A238
G22
T298
T211
A86
A210
G162
T213
T231


Clone Rc14
0.22x10-4 mutn.bp-1.div-1
0
T232
G245
14 sequences with 1 to 2 mutations
A235 G245
G45
C231
T243

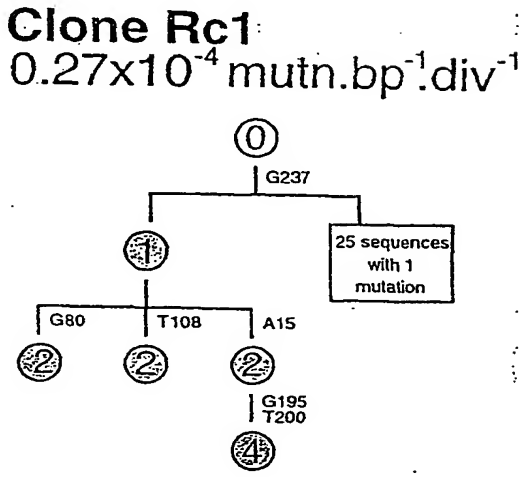

Clone Rc1
0.27x10-4 mutn.bp-1.div-1
0
G237
25 sequences with 1 mutation
G80
T108
A15
G195 T200

FIG. 2

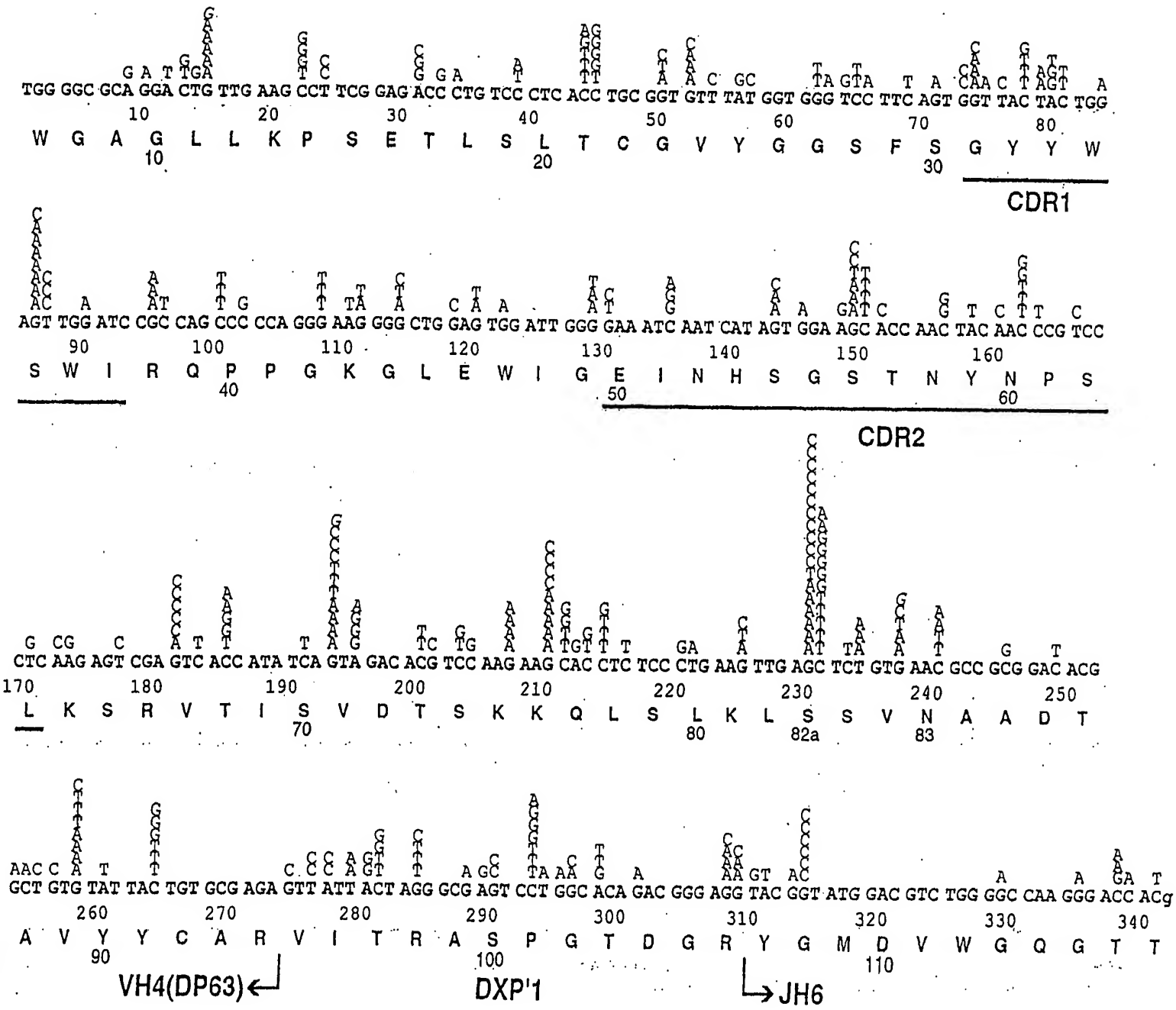

TGG GGC GCA GGA CTG TTG AAG CCT TCG GAG ACC CTG TCC CTC ACC TGC GGT GTT TAT GGT GGG TCC TTC AGT GGT TAC TAC TGG
W G A G L L K P S E T L S L T C G V Y G G S F S G Y Y W
CDR1
AGT TGG ATC CGC CAG CCC CCA GGG AAG GGG CTG GAG TGG ATT GGG GAA ATC AAT CAT AGT GGA AGC ACC AAC TAC AAC CCG TCC
S W I R Q P P G K G L E W I G E I N H S G S T N Y N P S
CDR2
CTC AAG AGT CGA GTC ACC ATA TCA GTA GAC ACG TCC AAG AAG CAC CTC TCC CTG AAG TTG AGC TCT GTG AAC GCC GCG GAC ACG
L K S R V T I S V D T S K K Q L S L K L S S V N A A D T
AAC GCT GTG TAT TAC TGT GCG AGA GTT ATT ACT AGG GCG AGT CCT GGC ACA GAC GGG AGG TAC GGT ATG GAC GTC TGG GGC CAA GGG ACC ACg
A V Y Y C A R V I T R A S P G T D G R Y G M D V W G Q G T T
VH4(DP63)
DXP'1
JH6

FIG. 3

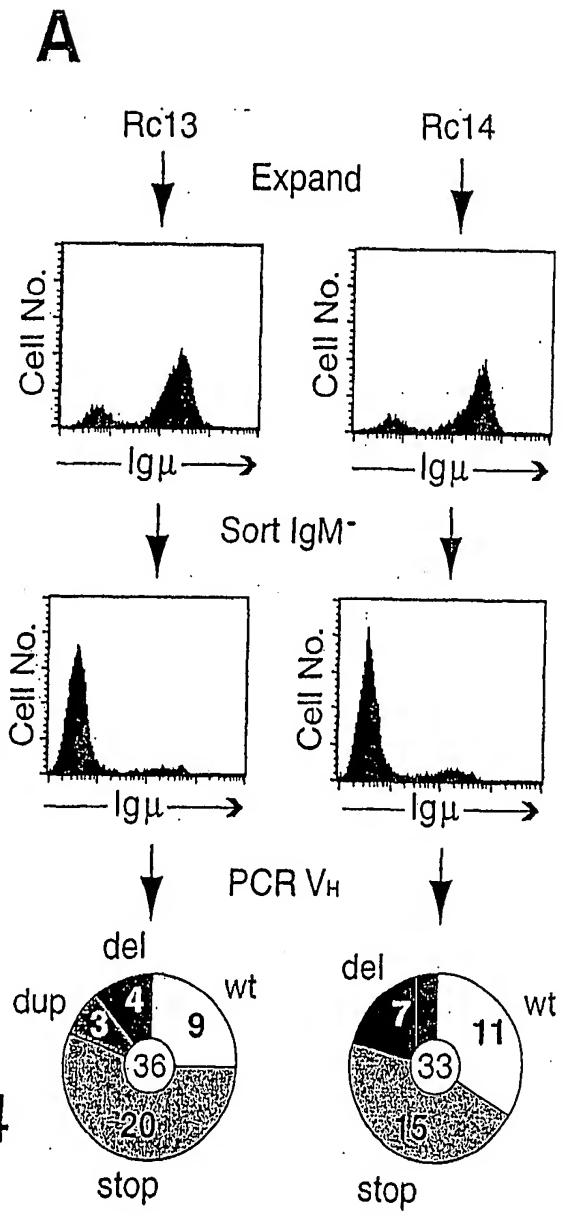


FIG. 4

B

	Rc13	Rc14
TAC 32	TAA(1;1)	TAA(1;1)
	TAG(5;2)	TAG(1;1)
TAC 33	TAG(2;2)	
TGG 34	TGA(1;1)	TGA(1;1)
TGG 36	TGA(6;5)	TGA(1;1)
GAG 46		TAG(1;1)
TGG 47	TAG(1;1)	TGA(1;1)
GAA 50		TAA(2;2)
TCA 70	TGA(2;1)	
TAT 90		TAA(3;2)
TAC 91	TAG(3;1)	TAG(2,2)
TAC106		TAA(1;1)

C

CLONE	% REVN.	STOP	REVN.
Rc13lo.1	1.2%	TAG32	TAC
Rc13lo.2	1.1%	TAG32	TAC TGG AAG
Rc13lo.3	0.08%	TGA36	TGG
Rc13lo.4	0.01%	TAA33	TAC

D

RGYW

TAG32 GGT**TAG**ATC

TGA36 AGT**TGA**ATC

TAA33 TAC**TAA**TGG

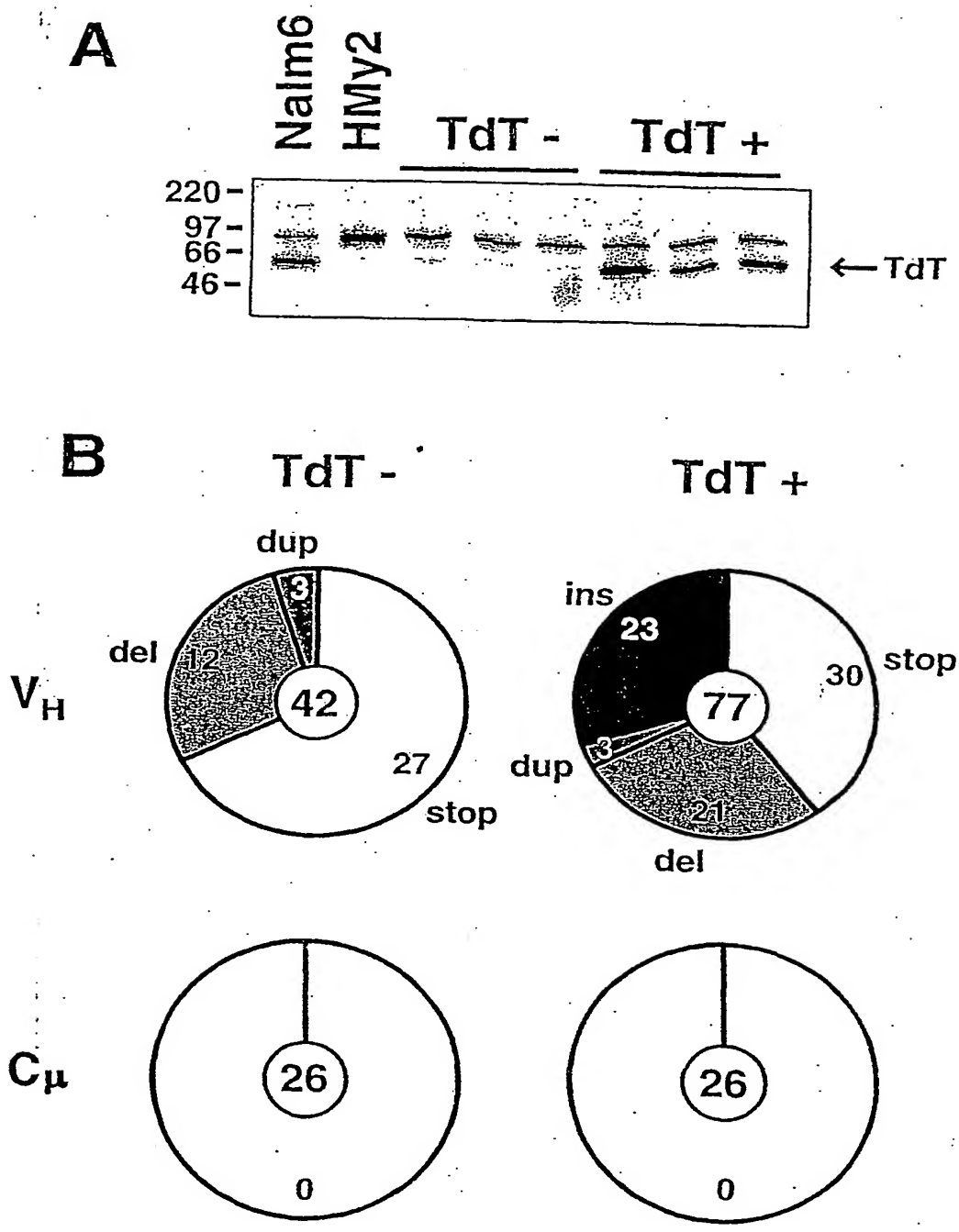

A
Nalm6
HMy2
TdT -
TdT +
220
97
66
46
←TdT
B
TdT -
TdT +
dup
3
del
12
42
27
stop
ins
23
30
stop
77
dup
3
21
del
V_H
C_μ
26
0
26
0

FIG. 5

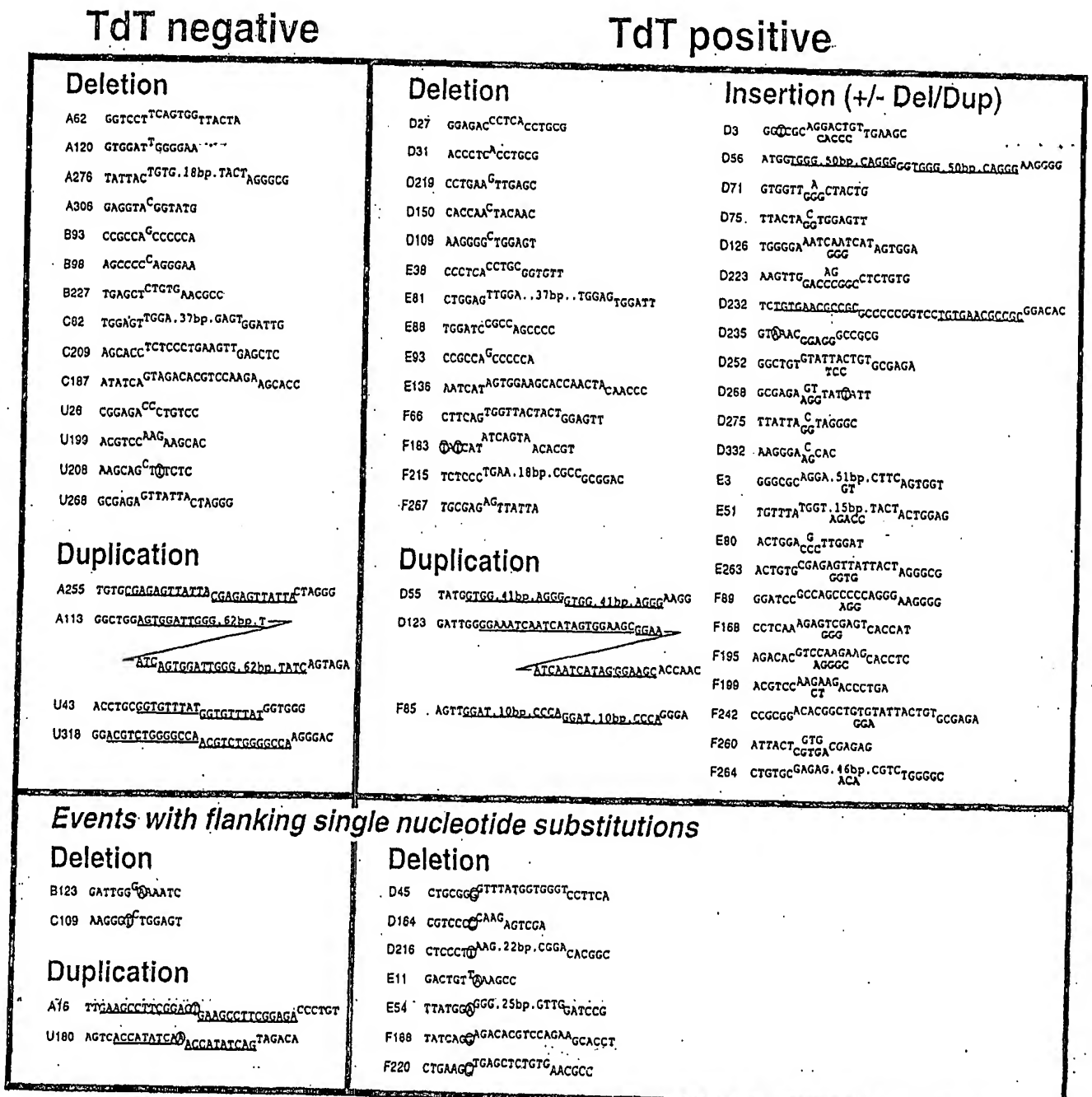

TdT negative
TdT positive
Deletion
A62 GGTCCT TCAGTGG TTACTA
A120 GTGGAT T GGGGAA
A276 TATTAC TGTG.18bp.TACT AGGGCG
A306 GAGGTA C GGTATG
B93 CCGCCA G CCCCCA
B98 AGCCCC C AGGGAA
B227 TGAGCT CTGTG AACGCC
C82 TGGAGT TGGA.37bp.GAGT GGATTG
C209 AGCACC TCTCCCTGAAGTT GAGCTC
C187 ATATCA GTAGACACGTCCAAGA AGCACC
U26 CGGAGA CC CTGTCC
U199 ACGTCC AAG AAGCAC
U208 AAGCAG C T TCTC
U268 GCGAGA GTTATTA CTAGGG
Duplication
A255 TGTGCGAGAGTTATTA CGAGAGTTATTA CTAGGG
A113 GGCTGG AGTGGATTGGG.62bp.T
ATC AGTGGATTGGG.62bp.TATC AGTAGA
U43 ACCTGC GGTGTTTAT GGTGTTTAT GGTGGG
U318 GG ACGTCTGGGGCCA ACGTCTGGGGCCA AGGGAC
Deletion
D27 GGAGAC CCTCA CCTGCG
D31 ACCCTC A CCTGCG
D219 CCTGAA G TTGAGC
D150 CACCAA C TACAAC
D109 AAGGGG C TGGAGT
E38 CCCTCA CCTGC GGTGTT
E81 CTGGAG TTGGA..37bp..TGGAG TGGATT
E88 TGGATC CGCC AGCCCC
E93 CCGCCA G CCCCCA
E136 AATCAT AGTGGAAGCACCAACTA CAACCC
F66 CTTCAG TGGTTACTACT GGAGTT
F183 ATCAT ATCAGTA ACACGT
F215 TCTCCC TGAA.18bp.CGCC GCGGAC
F267 TGCGAG AG TTATTA
Duplication
D55 TATGGTGG.41bp.AGGG GTGG.41bp.AGGG AAGG
D123 GATTGGGGAAATCAATCATAGTGGAAGC GGAA
ATCAATCATAG GGAAGC ACCAAC
F85 AGTTGGAT.10bp.CCCA GGAT.10bp.CCCA GGGA
Insertion (+/- Del/Dup)
D3 GG CGC AGGACTGT TGAAGC
CACCC
D56 ATGG TGGG.50bp.CAGGG GGTGGG.50bp.CAGGG AAGGGG
D71 GTGGTT A GGG CTACTG
D75. TTACTA C GG TGGAGTT
D126 TGGGGA AATCAATCAT AGTGGA
GGG
D223 AAGTTG AG GACCCGGC CTCTGTG
D232 TCTGTGAACGCCGC GCCCCCGGTCC TGTGAACGCCGC GGACAC
D235 GT AAC GGAGG GCCGCG
D252 GGCTGT GTATTACTGT GCGAGA
TCC
D268 GCGAGA GT AGG TAT ATT
D275 TTATTA C GG TAGGGC
D332 AAGGGA C AG CAC
E3 GGGCGC AGGA.51bp.CTTC AGTGGT
GT
E51 TGTTTA TGGT.15bp.TACT ACTGGAG
AGACC
E80 ACTGGA G CCC TTGGAT
E263 ACTGTG CGAGAGTTATTACT AGGGCG
GGTG
F89 GGATCC GCCAGCCCCCAGGG AAGGGG
AGG
F168 CCTCAA AGAGTCGAGT CACCAT
GGG
F195 AGACAC GTCCAAGAAG CACCTC
AGGGC
F199 ACGTCC AAGAAG ACCCTGA
CT
F242 CCGCGG ACACGGCTGTGTATTACTGT GCGAGA
GGA
F260 ATTACT GTG CGTGA CGAGAG
F264 CTGTGC GAGAG.46bp.CGTC TGGGGC
ACA
Events with flanking single nucleotide substitutions
Deletion
B123 GATTGG G AAATC
C109 AAGGGG T TGGAGT
Duplication
A16 TT GAAGCCTTCGGAG T GAAGCCTTCGGAGA CCCTGT
U180 AGTC ACCATATCA G ACCATATCAG TAGACA
Deletion
D45 CTGCGG G GTTTATGGTGGGT CCTTCA
D164 CGTCCC C CAAG AGTCGA
D216 CTCCCT T AAG.22bp.CGGA CACGGC
E11 GACTGT T AAGCC
E54 TTATGG G GGG.25bp.GTTG GATCCG
F188 TATCAG G AGACACGTCCAGAA GCACCT
F220 CTGAAG C TGAGCTCTGTG AACGCC

FIG. 6

1/1 31/11

TGG GGC GCA GGA CTG TTG AAG CCT TCG GAG ACC CTG TCC CTC ACC TGC GGT GTT TAT GGT
W G A G L L K P S E T L S L T C G V Y G

61/21 91/31

GGG TCC TTC AGT GGT TAC TAC TGG AGC TGG ATC CGC CAG CCC CCA GGG AAG GGG CTG GAG
G S F S G Y Y W S W I R Q P P G K G L E
AGT
S

121/41 151/51

TGG ATT GGG GAA ATC AAT CAT AGT GGA AGC ACC AAC TAC AAC CCG TCC CTC AAG AGT CGA
W I G E I N H S G S T N Y N P S L K S R

181/61 211/71

GTC ACC ATA TCA GTA GAC ACG TCC AAG AAG CAG CTC TCC CTG AAG TTG AGC TCT GTG AAC
V T I S V D T S K K H L S L K L S S V N
ATC CAC
M H

241/81 271/91

GCC GCG GAC ACG GCT GTG TAT TAC TGT GCG AGA GTT ATT ACT AGG GCG AGT CCT GGA ACA
A A D T A V Y Y C A R V I T R A S P G T
TCG ACG CAT GGC
S T H G

301/101 331/111

GAC GGG AGG TAC GGT ATG GAC GTC TGG GGC CAA GGG ACC ACG
D G R Y G M D V W G Q G T T
GTT
V

FIG. 7

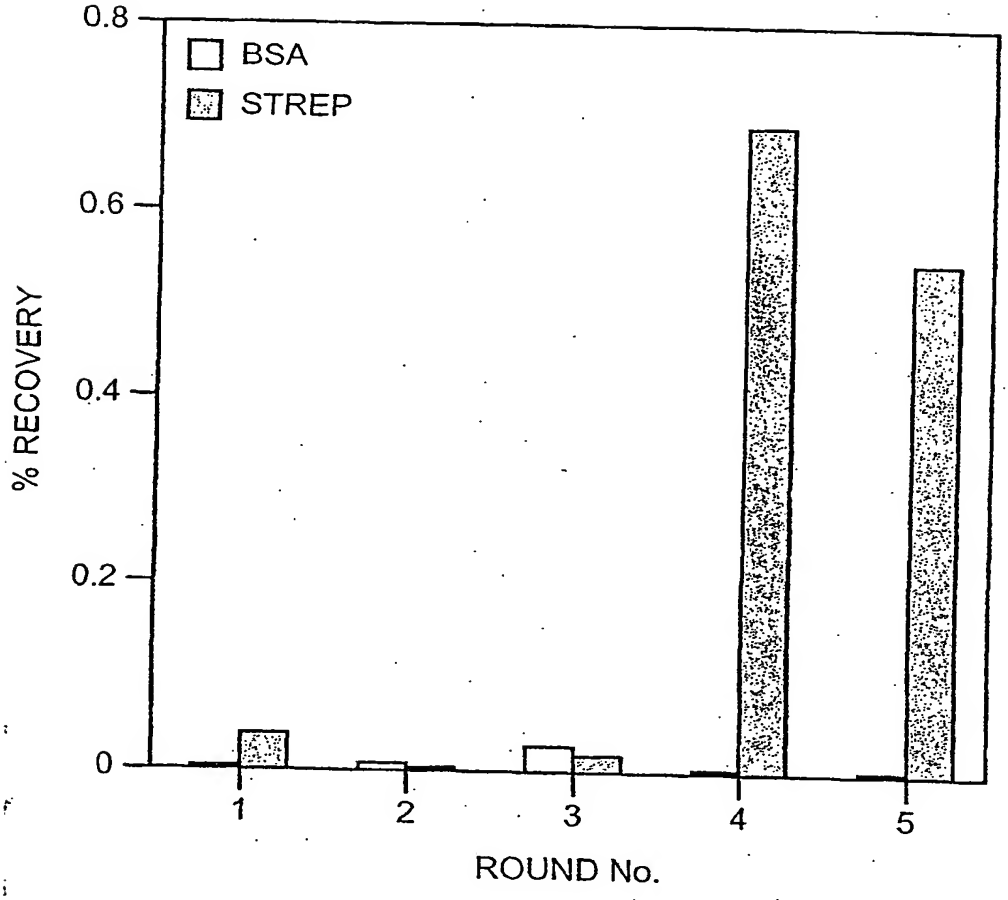


FIG. 8

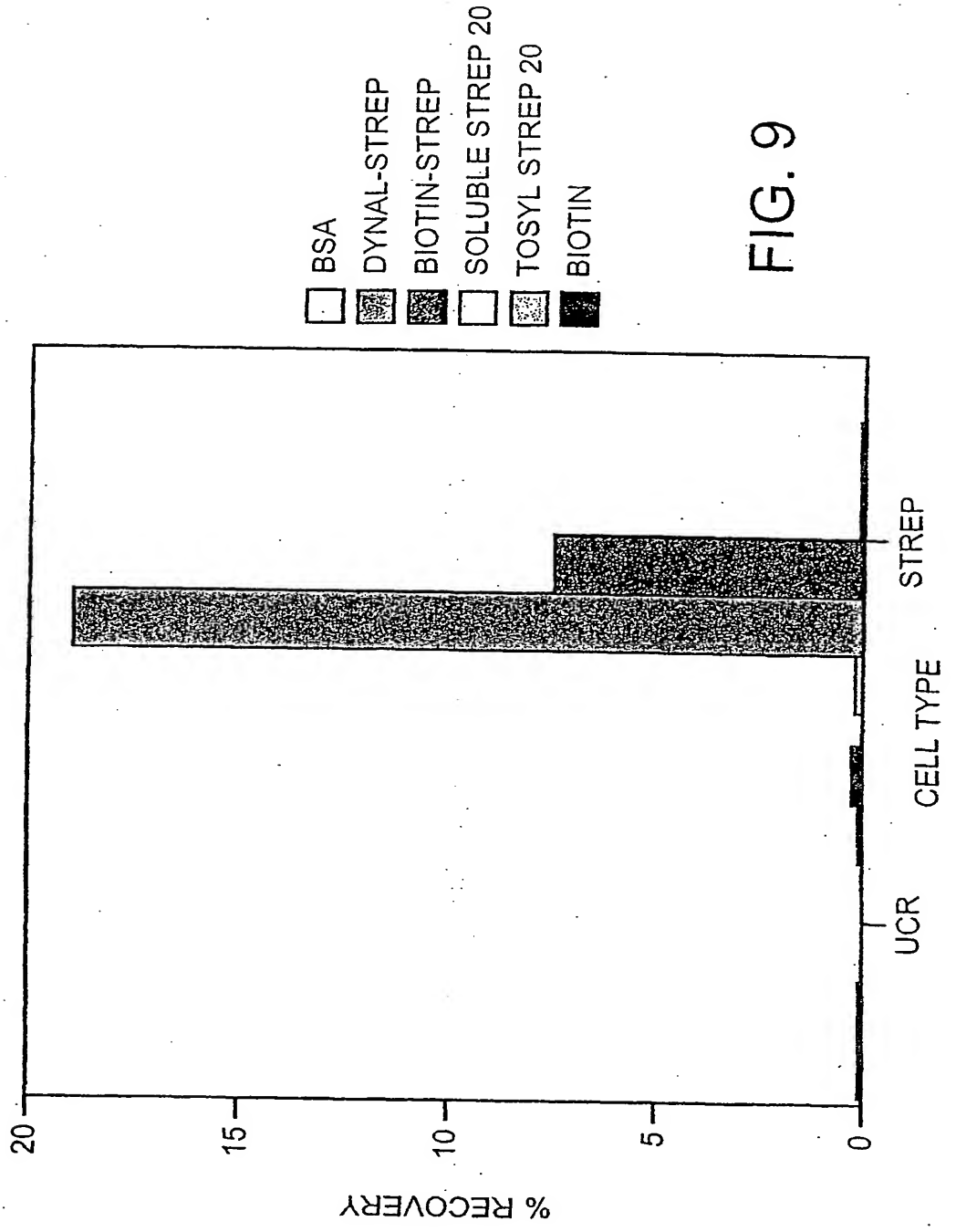

BSA
DYNAL-STREP
BIOTIN-STREP
SOLUBLE STREP 20
TOSYL STREP 20
BIOTIN
% RECOVERY
20
15
10
5
0
UCR
STREP
CELL TYPE

FIG. 9

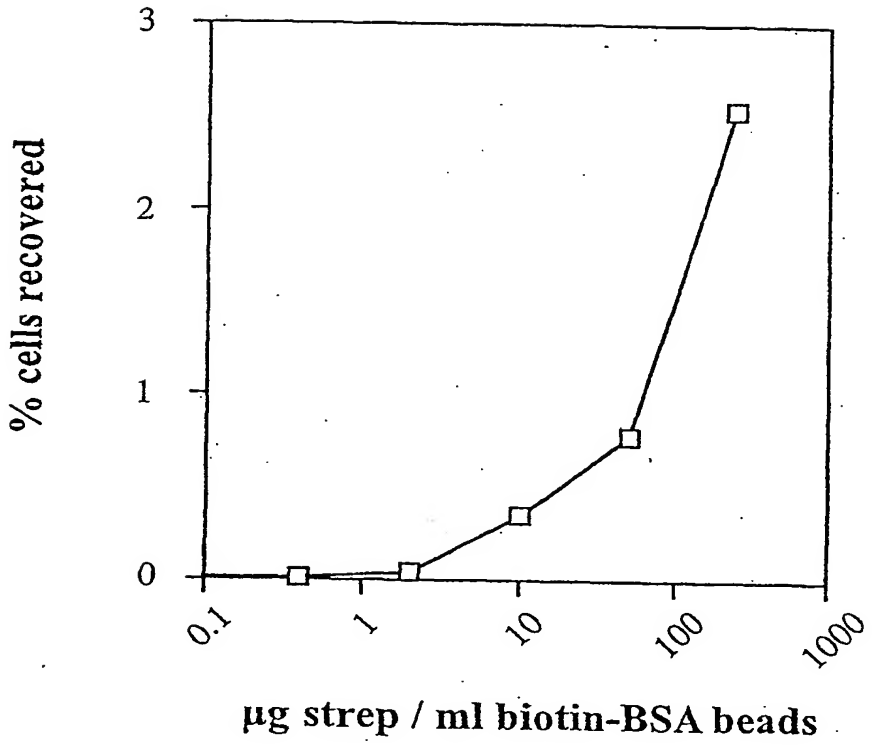

% cells recovered
3
2
1
0
0.1
1
10
100
1000
µg strep / ml biotin-BSA beads

FIG. 10

FIG. 11

VH

1/1

TGG GGC GCA GGA CTG TTG AAG CCT TCG GAG
W G A G L L K P S E

31/11

ACC CTG TCC CTC ACC TGC GGT GTT TAT GGT
T L S L T C G V Y G

61/21

GGG TCC TTC AGT GGT TAC TAC TGG AGC TGG
G S F S G Y Y W S W
GGA
G
AGT
S
ATT
I

91/31

ATC CGC CAG CCC CCA GGG AAG GGG CTG GAG
I R Q P P G K G L E

121/41

TGG ATT GGG GAA ATC AAT CAT AGT GGA AGC
W I G E I N H S G S

151/51

ACC AAC TAC AAC CCG TCC CTC AAG AGT CGA
T N Y N P S L K S R

181/61

GTC ACC ATA TCA GTA GAC ACG TCC AAG AAG
V T I S V D T S K K

211/71

CAG CTC TCC CTG AAG TTG AGC TCT GTG AAC
H L S L K L S S V N
CAC
H
AAC
N

241/81

GCC GCG GAC ACG GCT GTG TAT TAC TGT GCG
A A D T A V Y Y C A

271/91

AGA GTT ATT ACT AGG GCG AGT CCT GGA ACA
R V I T R A S P G T

301/101

GAC GGG AGG TAC GGT ATG GAC GTC TGG GGC
D G R Y G M D V W G
AGC
S

331/111

CAA GGG ACC ACG
Q G T T

VL

1/1 31/11

CCT GCC TCC GTG TCT GGG TCT CCT GGA CAG TCG ATC ACC ATC TCC TGC ACT GGA ACC AGC

P A S V S G S P G Q S I T I S C T G T S

TAT

Y

61/21 91/31

AGT GAC GTT GGT GGT TAT AAC TAT GTC TCC TGG TAC CAA CAA AAC CCA GGC AAA GCC CCC

S D V G G Y N Y V S W Y Q Q N P G K A P

TTT TGT

F C

121/41 151/51

AAA CTC ATG ATT TAT GAT GTC AGT AAT CGG CCC TCA GGG ATT TCT AAT CGC TTC TCT GGC

K L M I Y D V S N R P S G I S N R F G S

AAT CGA TTA

N R L

181/61 211/71

TCC AAG TCT GGC AAC ACG GCC TCC CTG ACC ATC TCT GGG CTC CAG GCT GAC GAC GAG GCT

S K S G N T A S L T I S G L Q A D D E A

ATC

I

241/81 271/91

GAT TAT TAC TGC ACC TCA TAT ACA AAC GAC AGC AAT TCT CAG GTA TTC GGC GGA GGG ACC

D Y Y C T S Y T N D S N S Q V F G G G T

ACT

T

FIG. 11CONT'D

FIG. 12

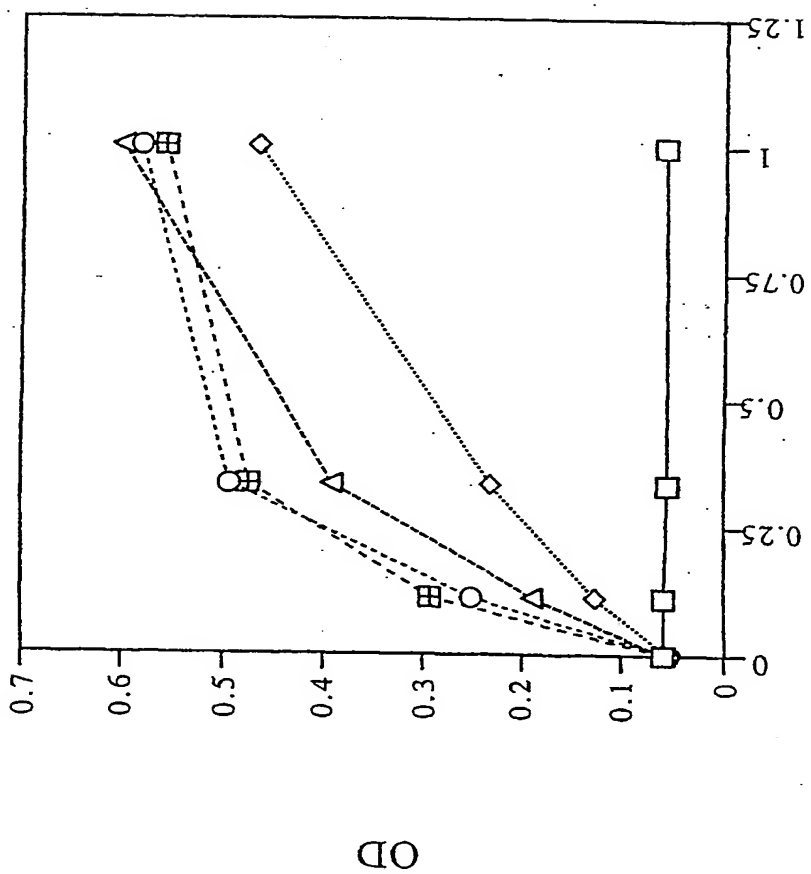

IgM in supernatant
Medium
Ramos
Round 4
Round 6
Round 7
OD
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
0.25
0.5
0.75
1
1.25
Dilution

FIG. 13

Streptavidin binding of Supernatants: ELISA

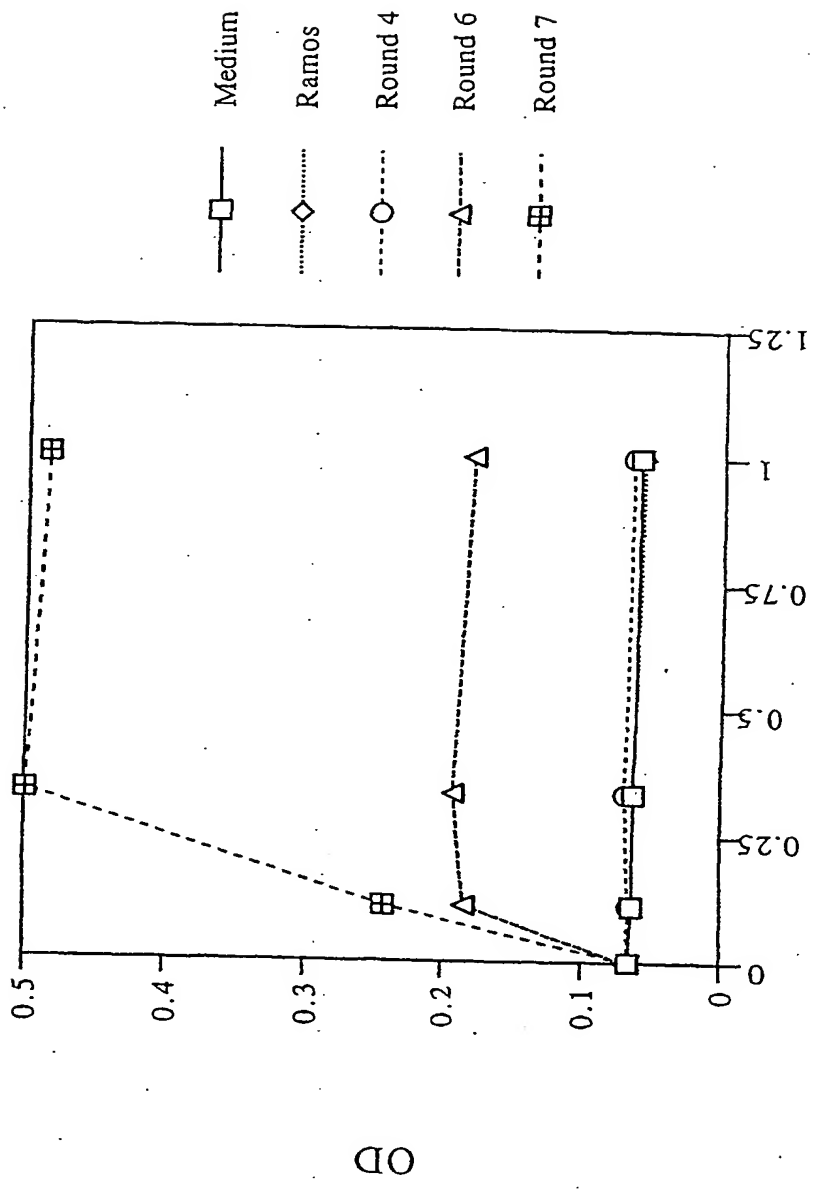
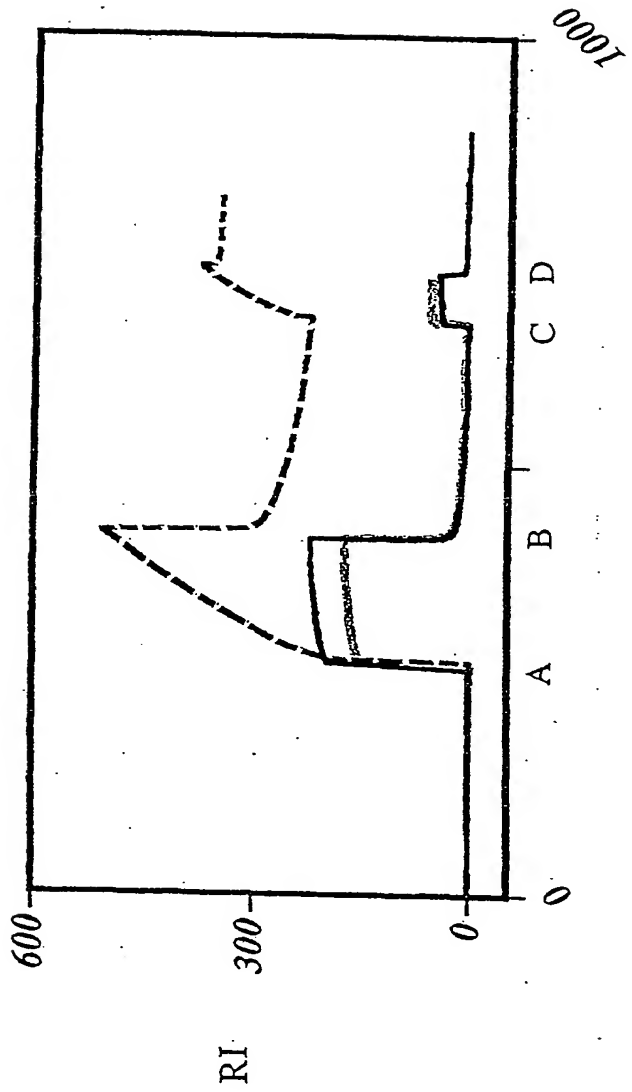


FIG. 14

Streptavidin binding of Supernatants: Biacore



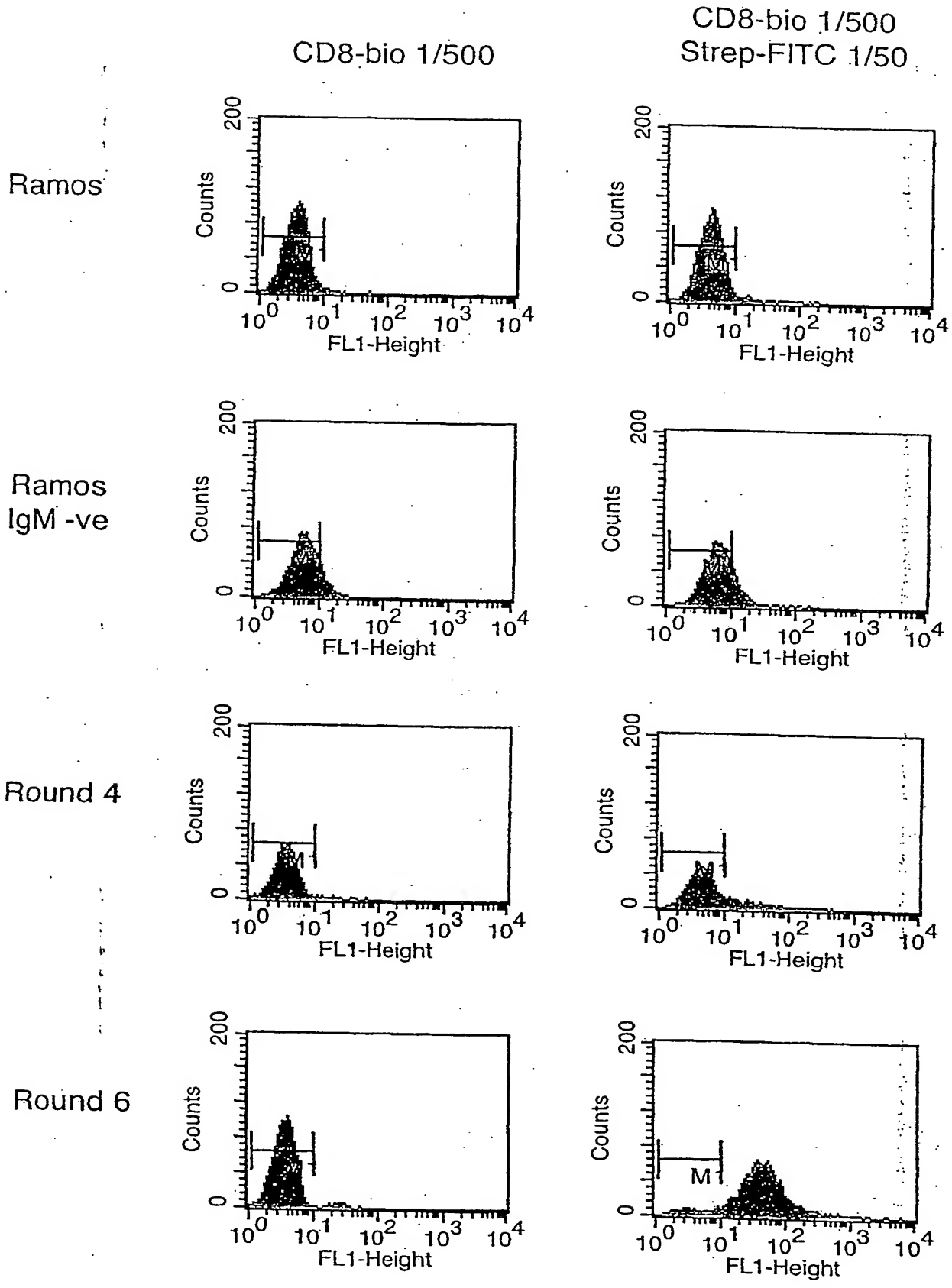

CD8-bio 1/500
CD8-bio 1/500
Strep-FITC 1/50
Ramos
Ramos
IgM -ve
Round 4
Round 6
Counts
FL1-Height
M1

FIG. 15

FIG. 16

VH

```
1/1                                                    31/11
TGG GGC GCA GGA CTG TTG AAG CCT TCG GAG ACC CTG TCC CTC ACC TGC GGT GTT TAT GGT
W   G   A   G   L   L   K   P   S   E   T   L   S   L   T   C   G   V   Y   G

61/21          CDR1                                    91/31
GGG TCC TTC AGT GGT TAC TAC TGG AGC TGG ATC CGC CAG CCC CCA GGG AAG GGG CTG GAG
G   S   F   S   G   Y   Y   W   S   W   I   R   Q   P   P   G   K   G   L   E

121/41         CDR2                                    151/51
TGG ATT GGG GAA ATC AAT CAT AGT GGA AGC ACC AAC TAC AAC CCG TCC CTC AAG AGT CGA
W   I   G   E   I   N   H   S   G   S   T   N   Y   N   P   S   L   K   S   R

181/61                                                 211/71
GTC ACC ATA TCA GTA GAC ACG TCC AAG AAG CAG CTC TCC CTG AAG TTG AGC TCT GTG AAC
V   T   I   S   V   D   T   S   K   K   H   L   S   L   K   L   S   S   V   N

241/81                                                 271/91  DJ
GCC GCG GAC ACG GCT GTG TAT TAC TGT GCG AGA GTT ATT ACT AGG GCG AGT CCT GGA ACA
A   A   D   T   A   V   Y   Y   C   A   R   V   I   T   R   A   S   P   G   T

301/101                                                331/111
GAC GGG AGG TAC GGT ATG GAC GTC TGG GGC CAA GGG ACC ACG
D   G   R   Y   G   M   D   V   W   G   Q   G   T   T
        AGC
        S
```

VL

1/1 31/11 CDR1

CCT GCC TCC GTG TCT GGG TCT CCT GGA CAG TCG ATC ACC ATC TCC TGC ACT GGA ACC AGC

P A S V S G S P G Q S I T I S C T G T S

61/21 91/31

AGT GAC GTT GGT GGT TAT AAC TAT GTC TCC TGG TAC CAA CAA AAC CCA GGC AAA GCC CCC

S D V G G Y N Y V S W Y Q Q N P G K A P

TTT TGT

F C

121/41 CDR2 151/51

AAA CTC ATG ATT TAT GAT GTC AGT AAT CGG CCC TCA GGG ATT TCT AAT CGC TTC TCT GGC

K L M I Y D V S N R P S G I S N R F G S

GCT

A

181/61 211/71

TCC AAG TCT GGC AAC ACG GCC TCC CTG ACC ATC TCT GGG CTC CAG GCT GAC GAC GAG GCT

S K S G N T A S L T I S G L Q A D D E A

241/81 CDR3 271/91

GAT TAT TAC TGC ACC TCA TAT ACA AAC GAC AGC AAT TCT CAG GTA TTC GGC GGA GGG ACC

D Y Y C T S Y T N D S N S Q V F G G G T

FIG. 16CONT'D

In Vitro Maturation

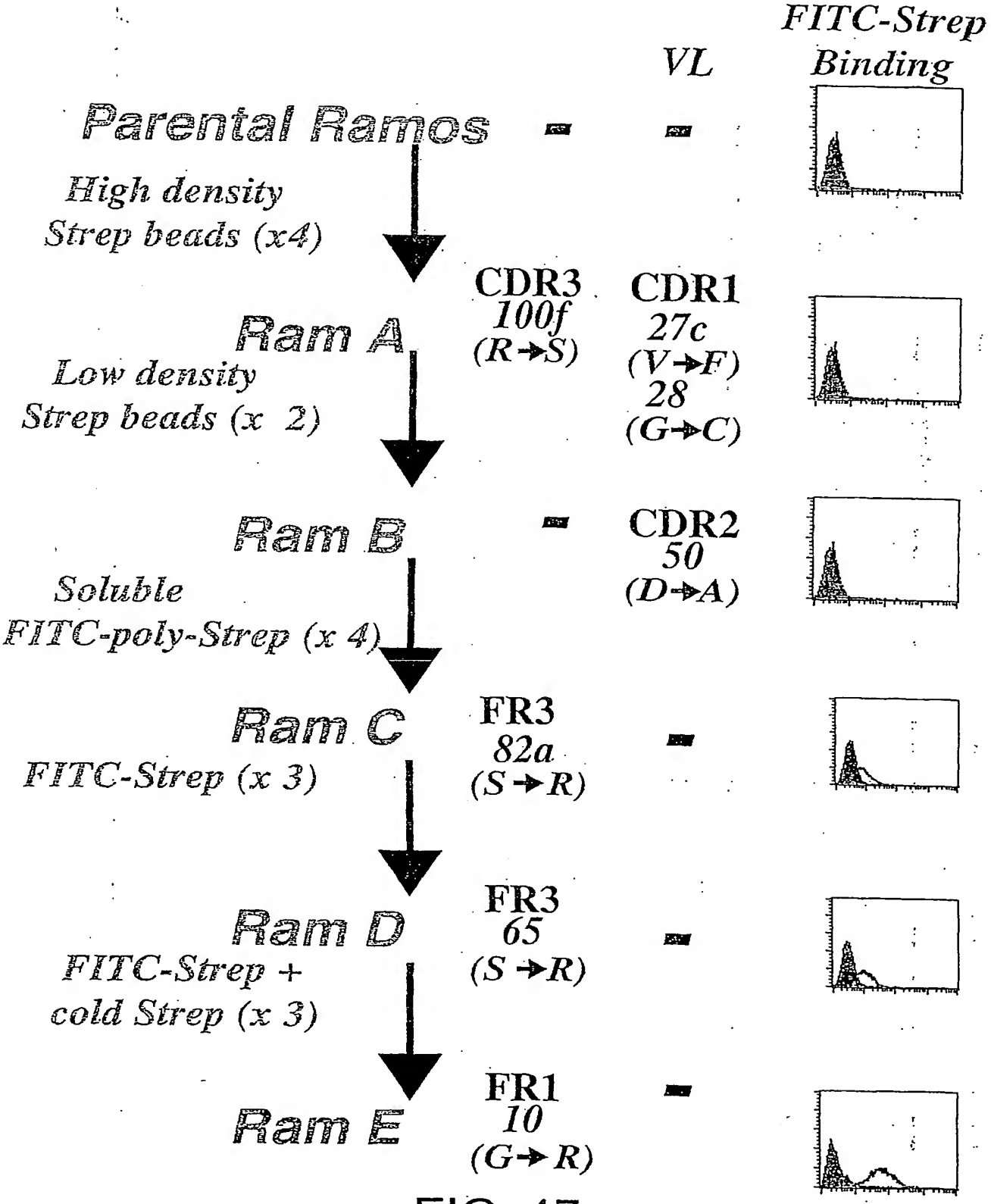


FIG. 17

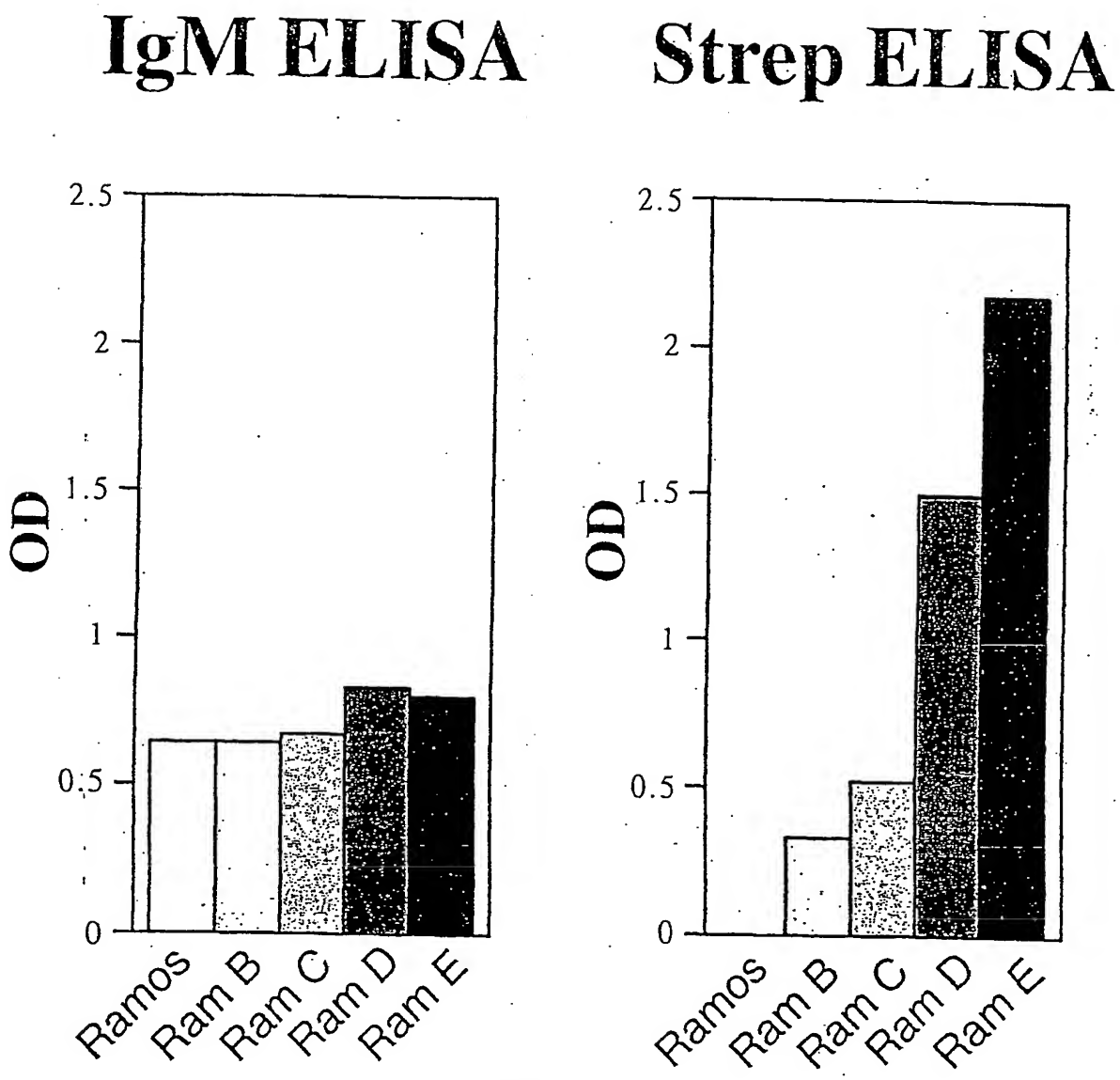

IgM ELISA
Strep ELISA
OD
2.5
2
1.5
1
0.5
0
Ramos
Ram B
Ram C
Ram D
Ram E

FIG. 18

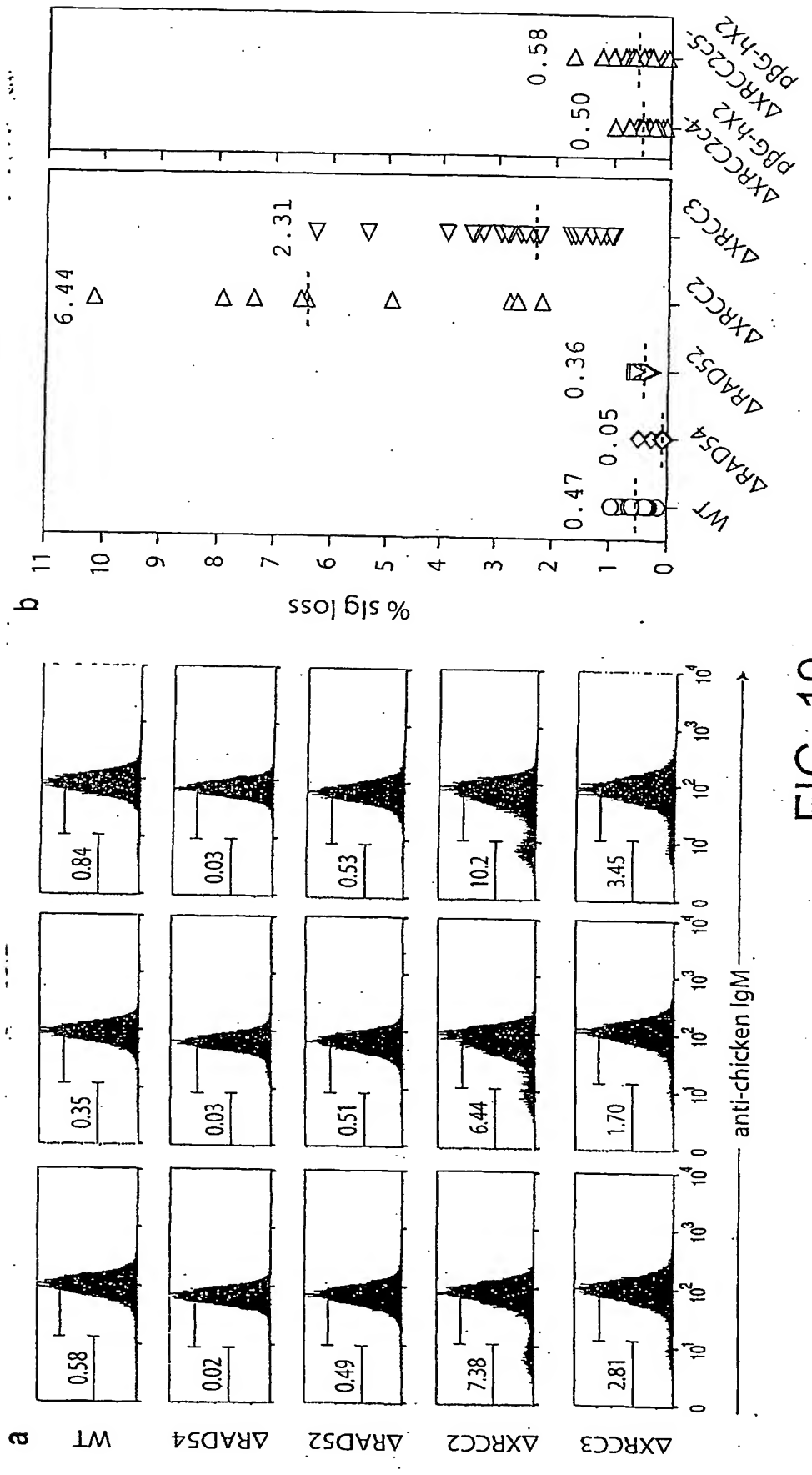

a
WT
ΔRAD54
ΔRAD52
ΔXRCC2
ΔXRCC3
0.58
0.35
0.84
0.02
0.03
0.03
0.49
0.51
0.53
7.38
6.44
10.2
2.81
1.70
3.45
anti-chicken IgM
b
% sIg loss
6.44
2.31
0.47
0.05
0.36
0.58
0.50
WT
ΔRAD54
ΔRAD52
ΔXRCC2
ΔXRCC3
ΔXRCC2A-pBG-hX2
ΔXRCC2S-pBG-hX2

FIG. 19

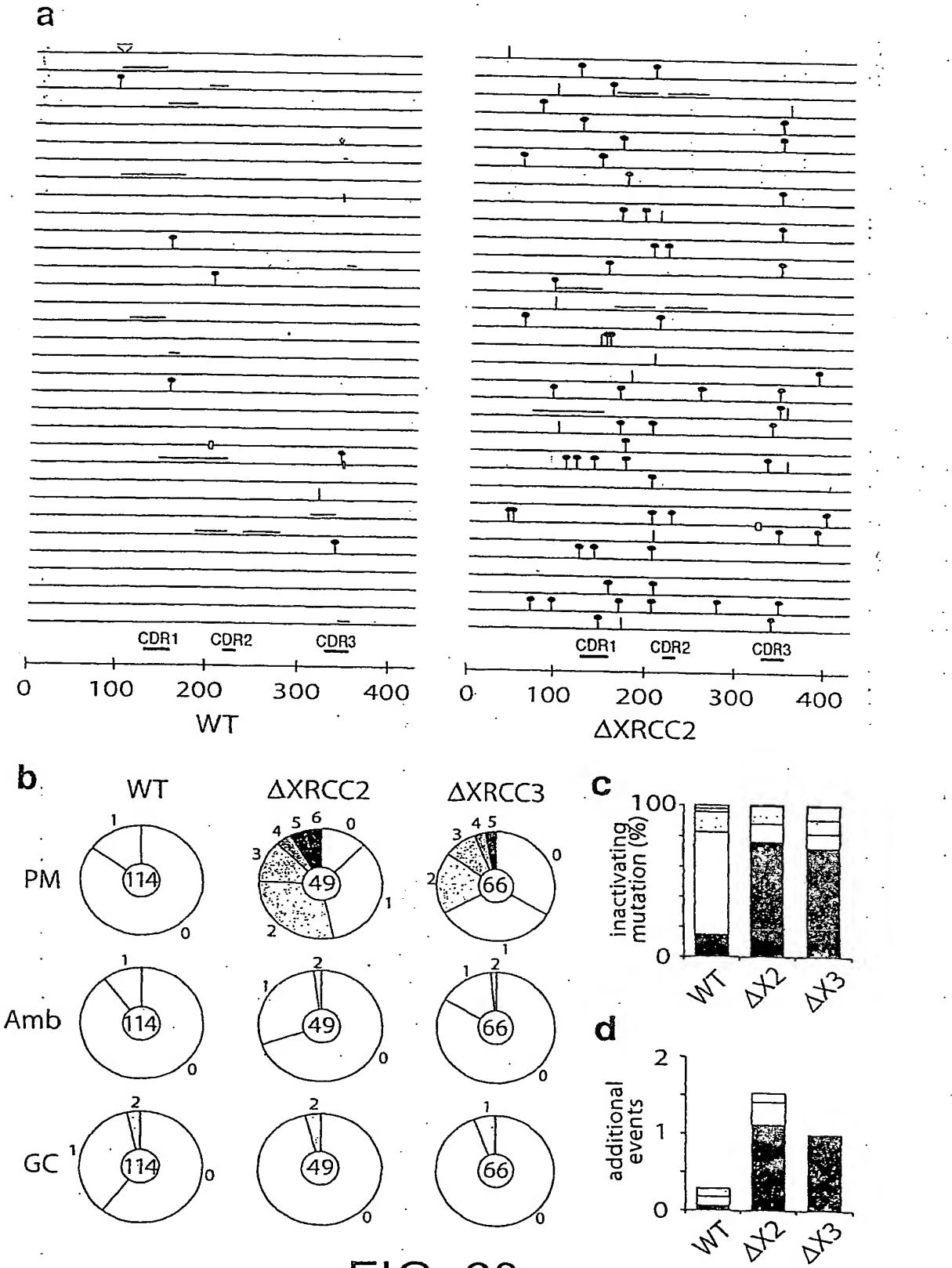

a
CDR1
CDR2
CDR3
0
100
200
300
400
WT
ΔXRCC2
b
WT
ΔXRCC2
ΔXRCC3
PM
Amb
GC
114
49
66
c
inactivating mutation (%)
100
0
WT
ΔX2
ΔX3
d
additional events
2
1
0

FIG. 20

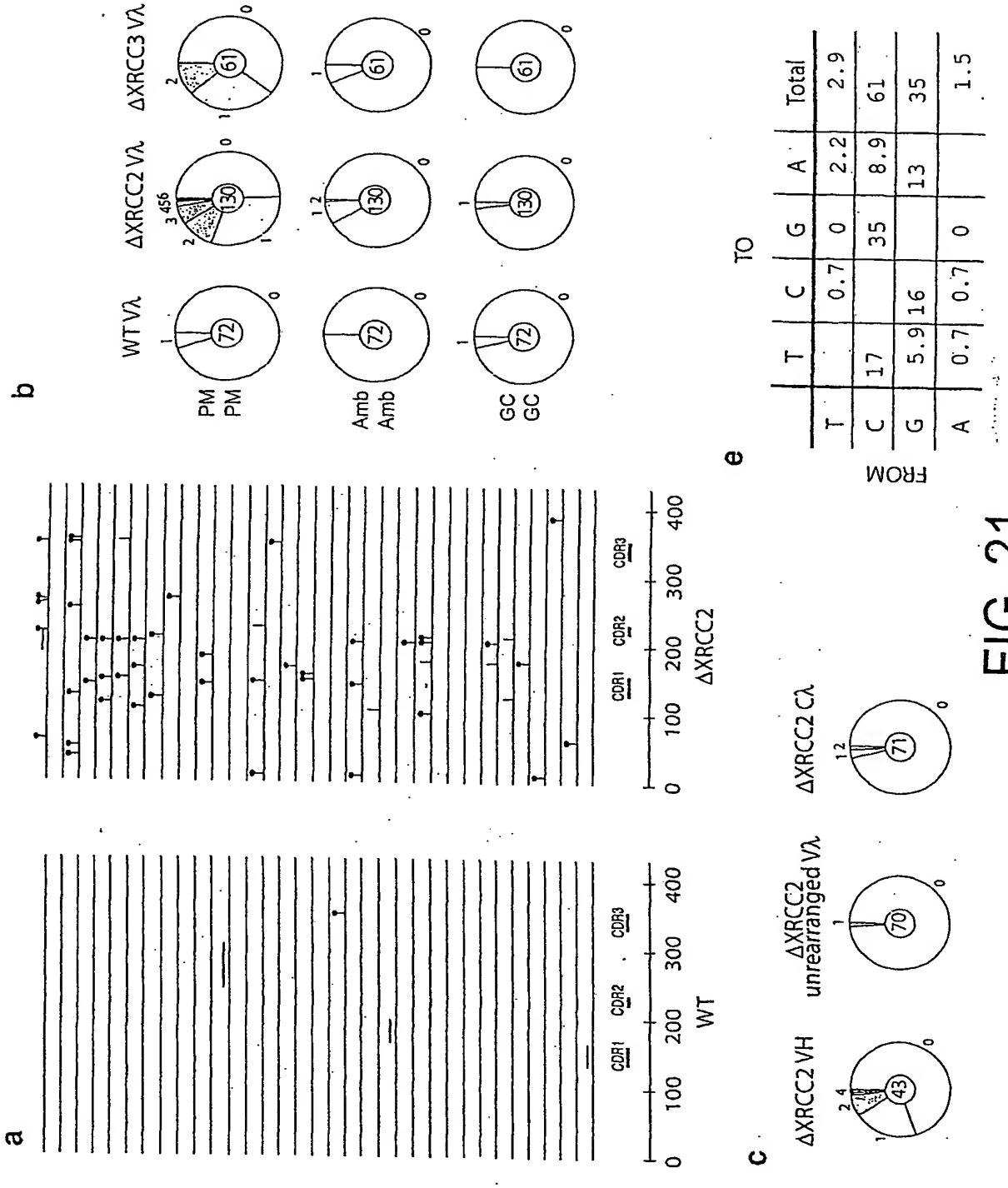

a
WT
ΔXRCC2
CDR1
CDR2
CDR3
0
100
200
300
400
b
WT Vλ
ΔXRCC2 Vλ
ΔXRCC3 Vλ
PM
PM
Amb
Amb
GC
GC
c
ΔXRCC2 VH
ΔXRCC2 unrearranged Vλ
ΔXRCC2 Cλ
e
FROM
TO
T
C
G
A
Total
T 0.7 2.2 2.9
C 17 35 8.9 61
G 5.9 16 13 35
A 0.7 0.7 0 1.5

FIG. 21

d

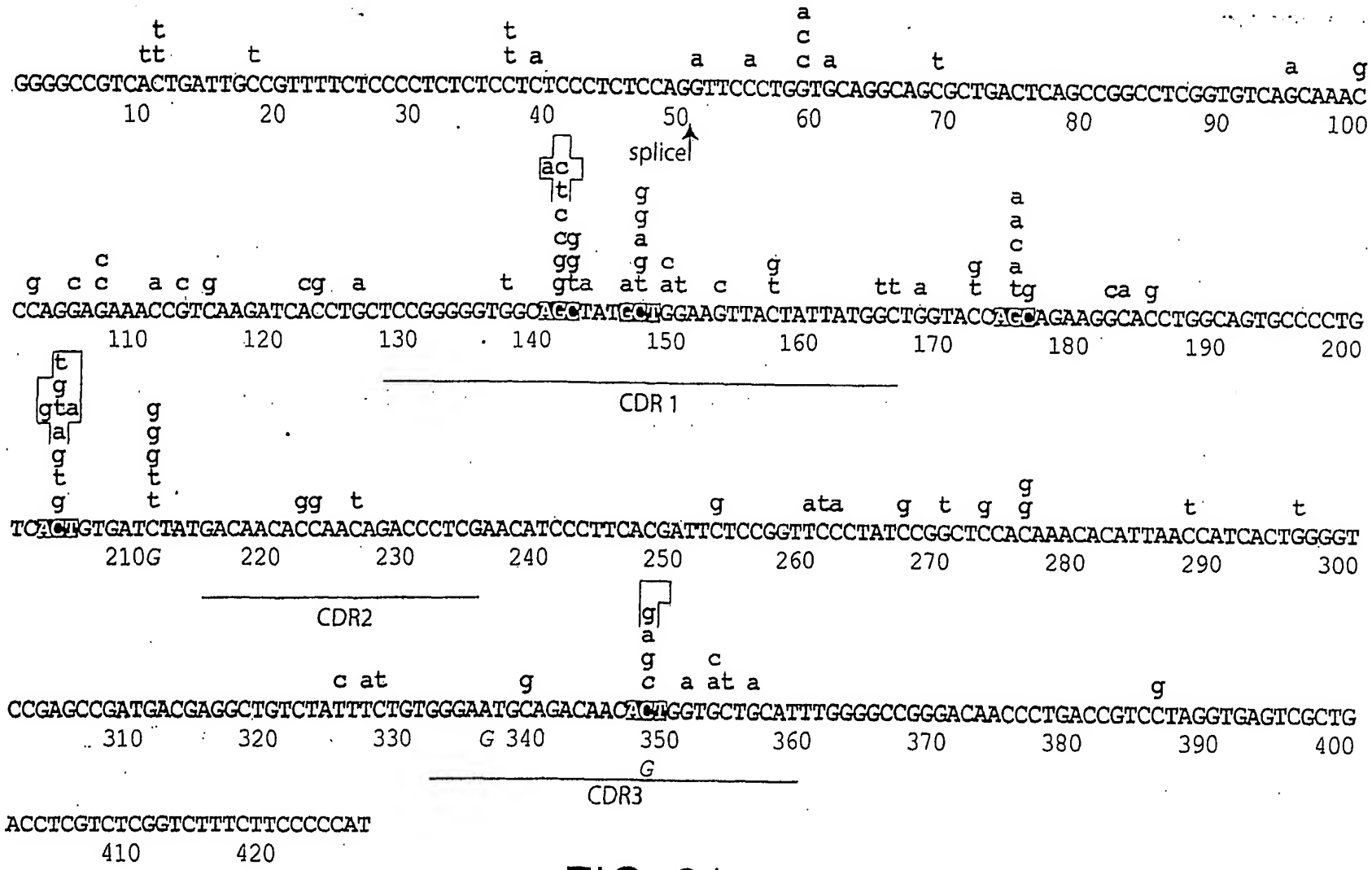


FIG. 21 CONT'D

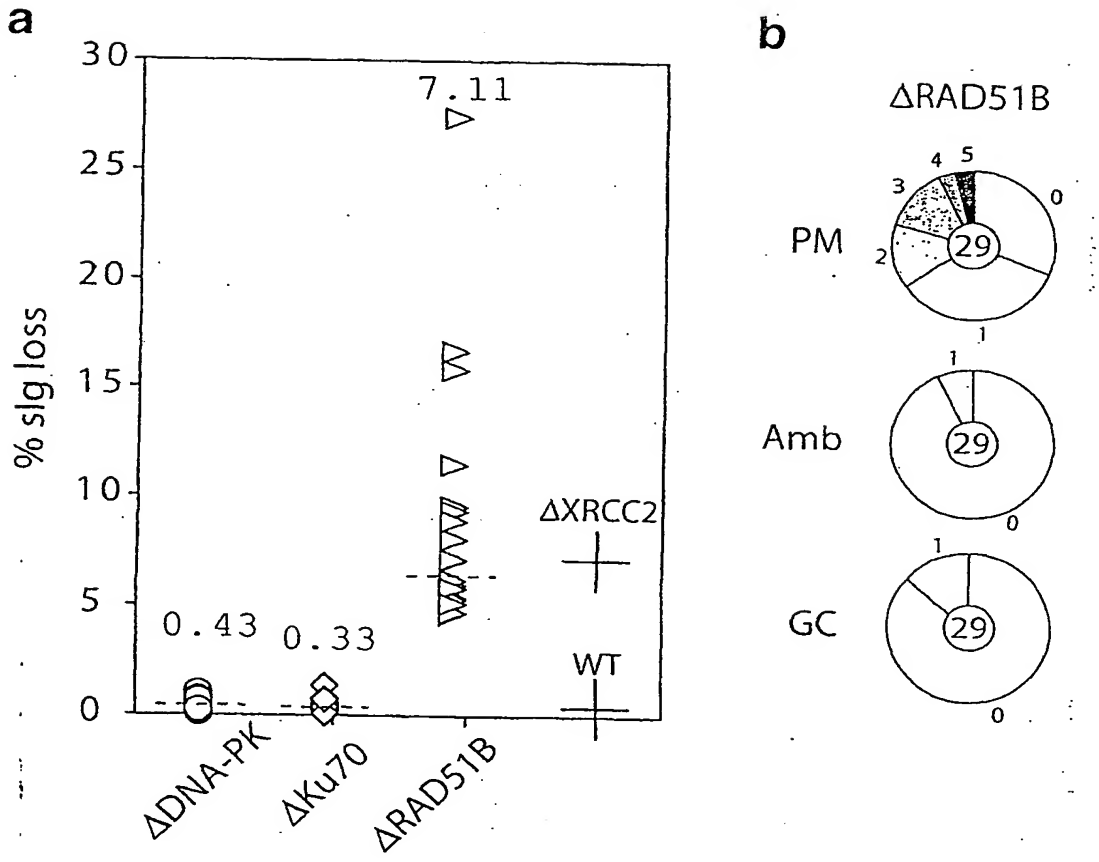

a
b
% slg loss
30
25
20
15
10
5
0
7.11
0.43
0.33
ΔXRCC2
WT
ΔDNA-PK
ΔKu70
ΔRAD51B
ΔRAD51B
PM
Amb
GC
29
0
1
2
3
4
5

FIG. 22

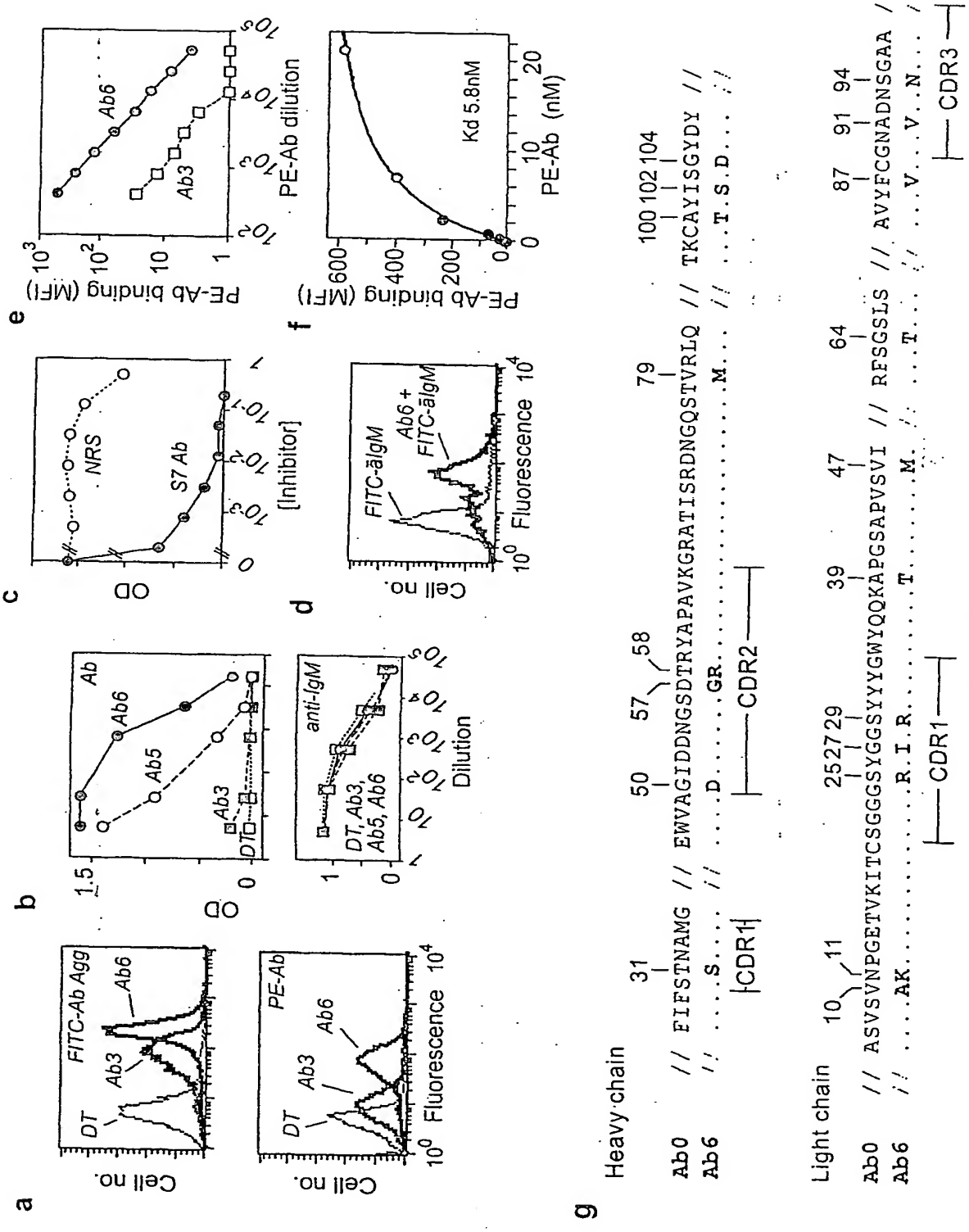

a
Cell no.
FITC-Ab Agg
Ab6
Ab3
DT
PE-Ab
Ab6
Ab3
DT
Fluorescence
b
OD
Ab
Ab6
Ab5
Ab3
DT
anti-IgM
DT, Ab3, Ab5, Ab6
Dilution
c
OD
NRS
S7 Ab
[Inhibitor]
d
Cell no.
FITC-αIgM
Ab6 + FITC-αIgM
Fluorescence
e
PE-Ab binding (MFI)
Ab6
Ab3
PE-Ab dilution
f
PE-Ab binding (MFI)
Kd 5.8nM
PE-Ab (nM)
g
Heavy chain
Ab0 // EIFSTNAMG // EWVAGIDDNGSDTRYAPAVKGRATISRDNGQSTVRLQ // TKCAYISGYDY //
Ab6 // ....S.... // ....D.....GR.....M.... // ...T.S.D... //
CDR1
CDR2
Light chain
Ab0 // ASVSVNPGETVKITCSGGGSYGGSYYYGWYQQKAPGSAPVSVI // RFSGSIS // AVYFCGNADNSGAA //
Ab6 // ....AK.....R.I.R.....T.....M. // ...T... // ...V...V..N... //
CDR1
CDR3

FIG. 23

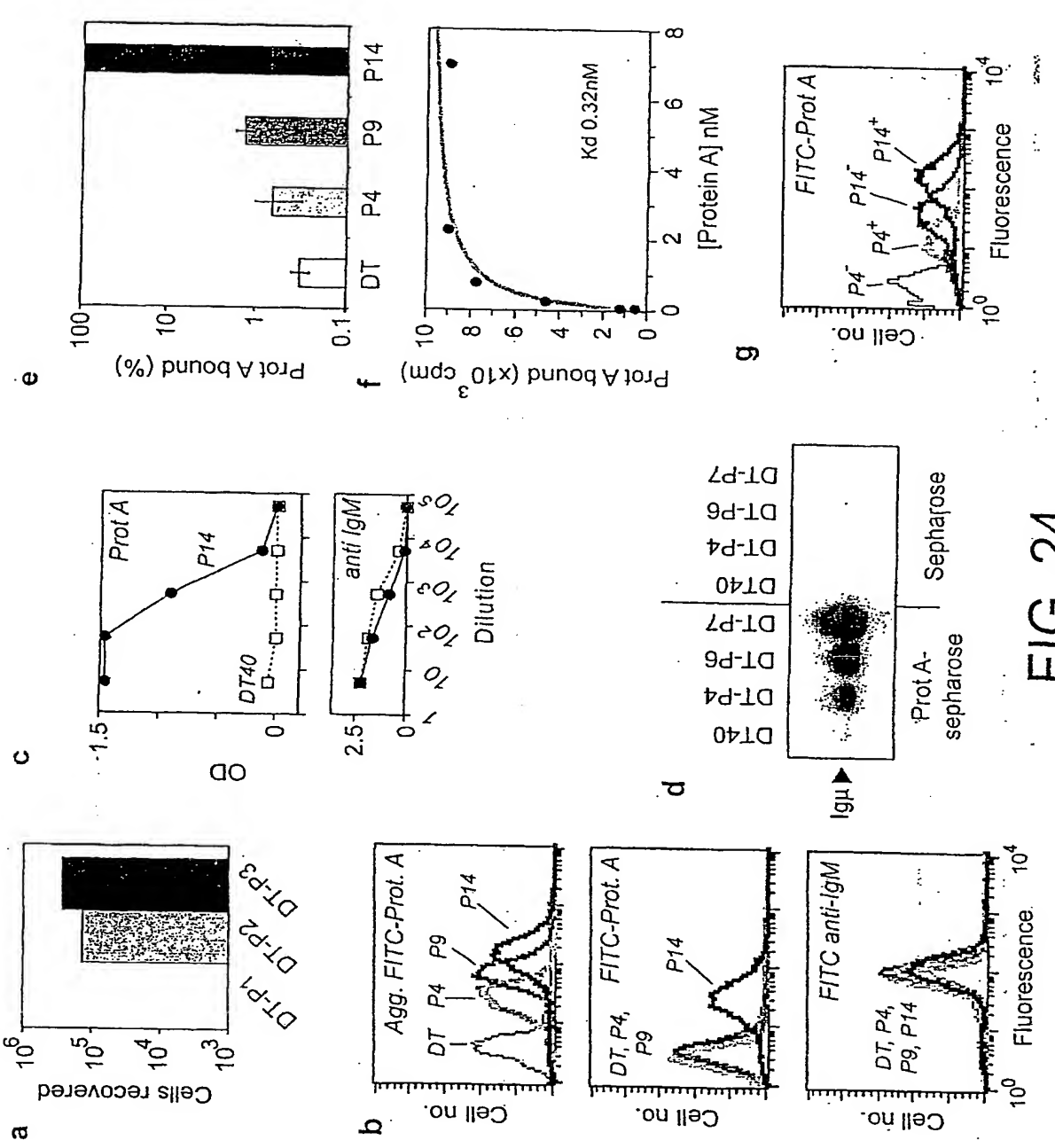

a
Cells recovered
DT-P1
DT-P2
DT-P3
b
Agg. FITC-Prot. A
DT
P4
P9
P14
FITC-Prot. A
DT, P4, P9
P14
FITC anti-IgM
DT, P4, P9, P14
Cell no.
Fluorescence
c
Prot A
P14
DT40
OD
anti IgM
Dilution
d
DT40
DT-P4
DT-P6
DT-P7
Igμ
Prot A-sepharose
Sepharose
e
Prot A bound (%)
DT
P4
P9
P14
f
Prot A bound (x10³ cpm)
Kd 0.32nM
[Protein A] nM
g
FITC-Prot A
P4⁻
P4⁺
P14⁻
P14⁺
Cell no.
Fluorescence

FIG. 24

h

```
Heavy chain
            17 19       31          42      54 57        68        77 80       102 105
            |  |        |           |       |  |         |         |  |        |  |
P0       // PGGALSLV // STNAMGWVRQAPDK // DNGSDTRYAPAVKGRATISRDNGQSTVRLQ // AYISGYDY //
P4       // ...P.R.. // .............. // .D......................R..... // .....C.. //
P6       // ...P.R.. // .............. // .D......................R..S.. // .....C.. //
P8,9,10  // ...P.R.. // .S..........G. // .D..G...................R..S.. // ..S..C.. //
P14      // ...P.R.. // .S..........G. // .D..G..........V........R..S.. // ..S..C.. //
                        |CDR1|            |---- CDR2 ----|

Light chain
                 10             25 28   33           46         94
                 |              |  |    |            |          |
P0       // ASVSVNPGETVKITCSGGGSYGGSYYYGWYQQKAPGSAPVSVI // DDEAVYFCGNADNSGAAFGA //
P4,6     // ...................R....................... // .............T...... //
P8       // ....A..............R..A....V............... // .............T...... //
P9,10,14 // ....A..............R..A....V............T.. // .............T...... //
                           |--- CDR1 ---|                   |- CDR3 -|
```

FIG.24 CONT'D

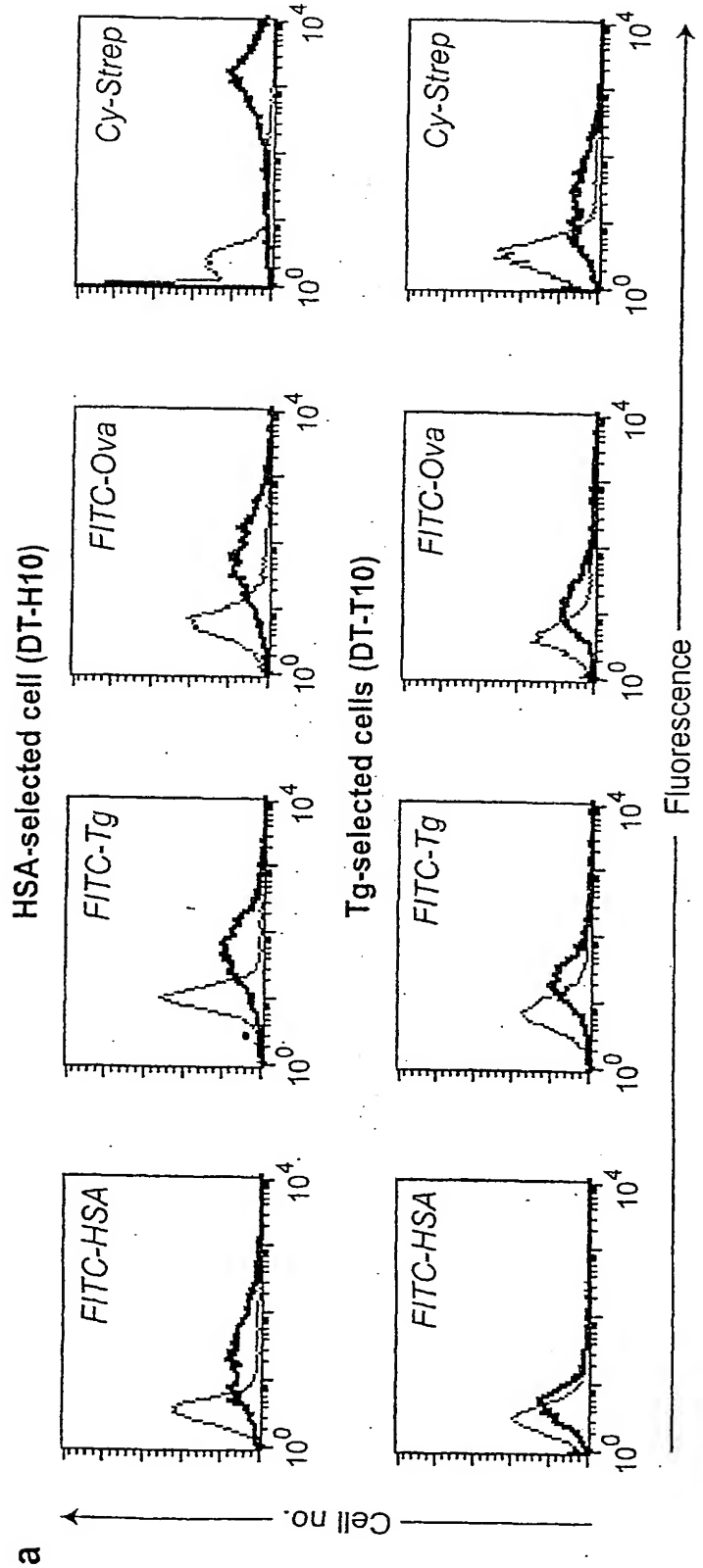

a
Cell no.
HSA-selected cell (DT-H10)
Tg-selected cells (DT-T10)
FITC-HSA
FITC-Tg
FITC-Ova
Cy-Strep
Fluorescence
10^0
10^4

FIG. 25

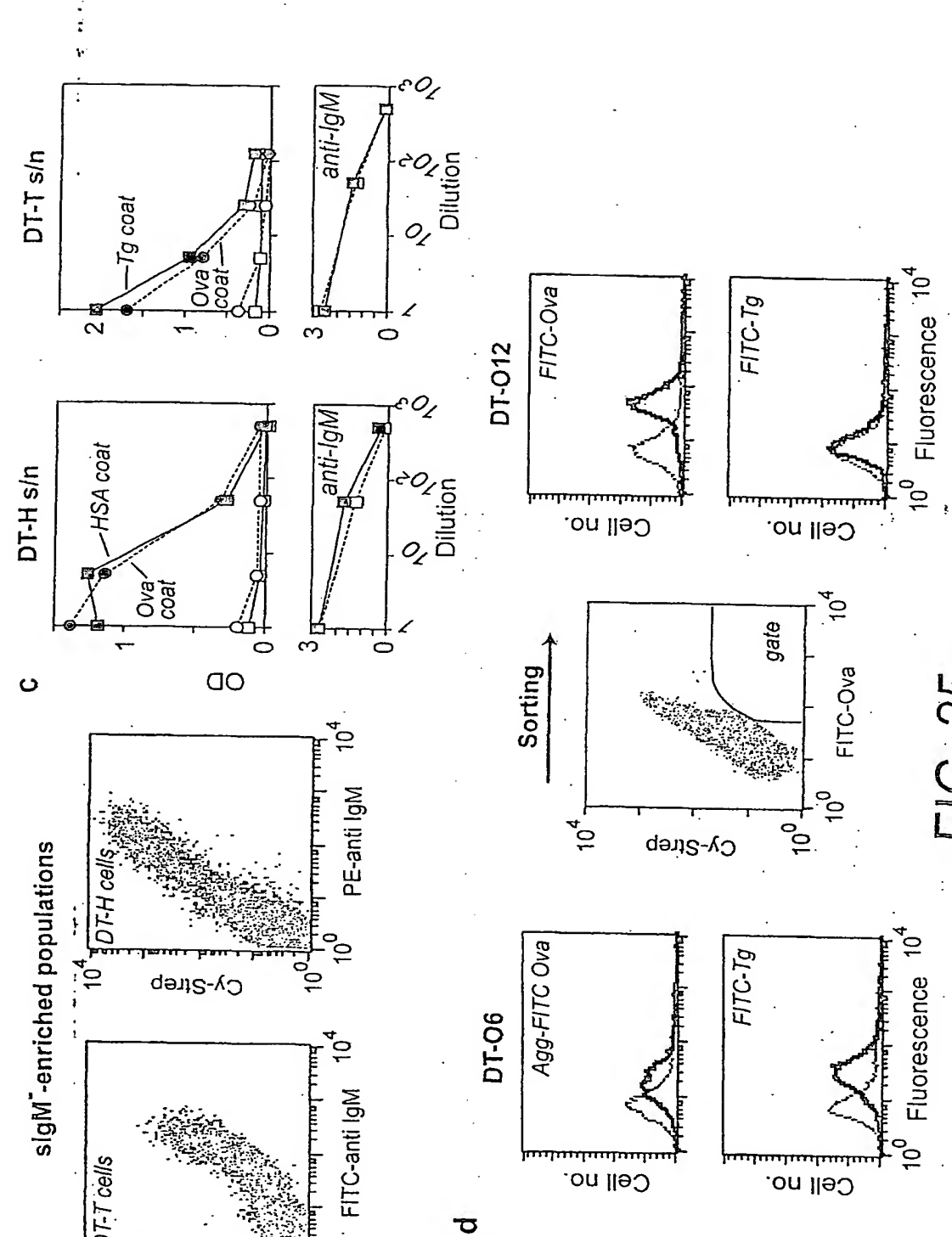

b
sIgM⁻-enriched populations
DT-T cells
Cy-strep/bio-Tg
FITC-anti IgM
DT-H cells
Cy-Strep
PE-anti IgM
c
DT-H s/n
HSA coat
Ova coat
OD
anti-IgM
Dilution
DT-T s/n
Tg coat
Ova coat
anti-IgM
Dilution
d
DT-O6
Agg-FITC Ova
FITC-Tg
Cell no.
Fluorescence
Sorting
Cy-Strep
FITC-Ova
gate
DT-O12
FITC-Ova
FITC-Tg
Cell no.
Fluorescence

FIG. 25CONT'D

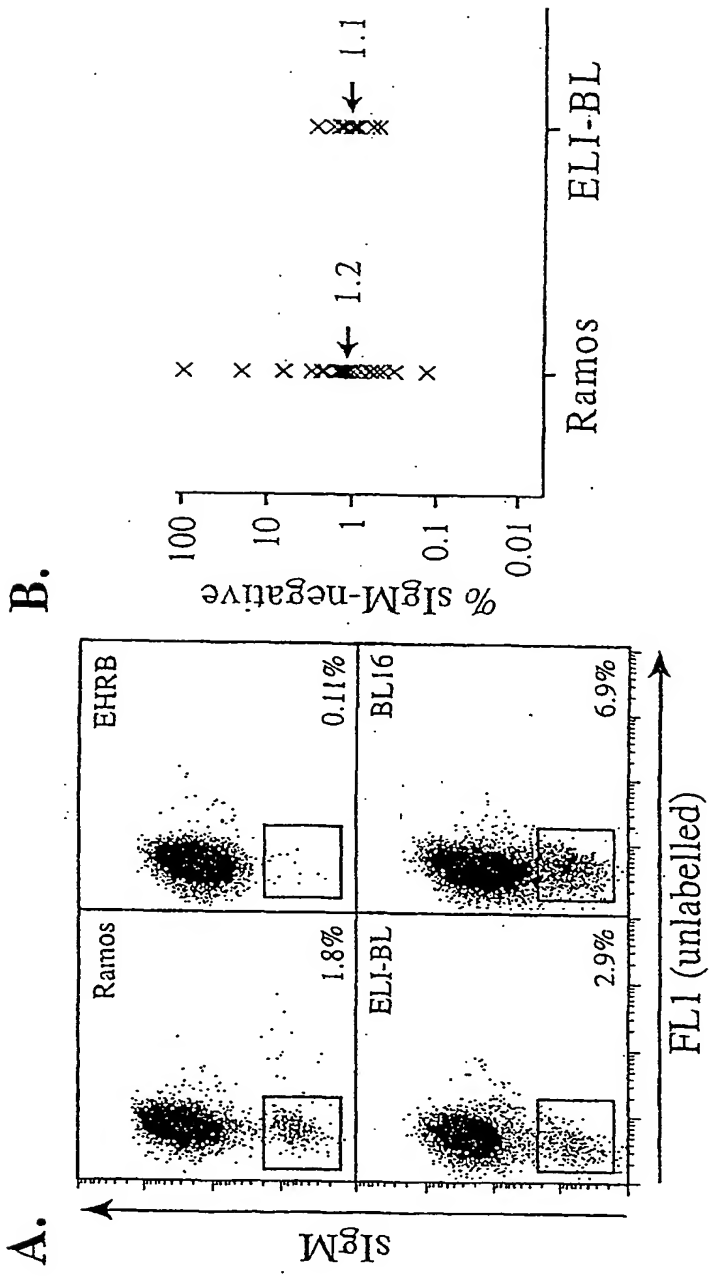

A.
sIgM
FL1 (unlabelled)
Ramos
1.8%
EHRB
0.11%
ELI-BL
2.9%
BL16
6.9%
B.
% sIgM-negative
100
10
1
0.1
0.01
1.2
1.1
Ramos
ELI-BL

FIG. 26

C.

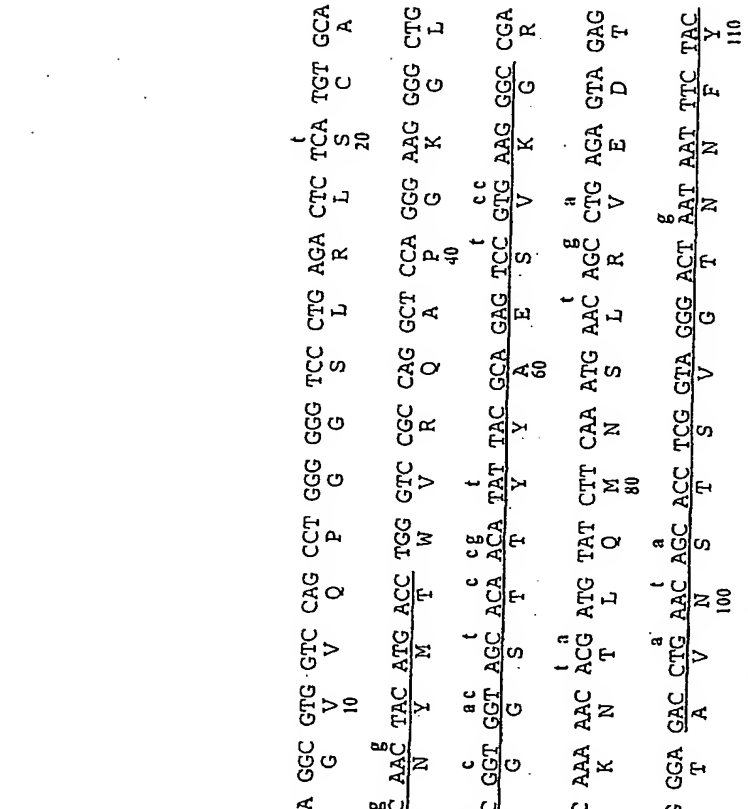


FIG. 26CONT'D